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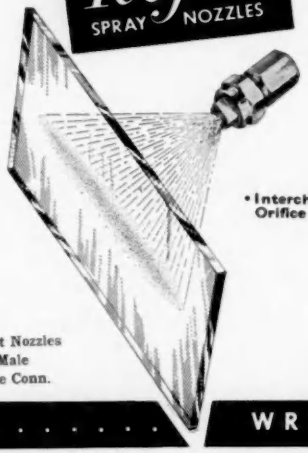

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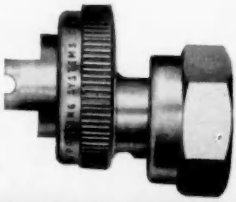

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TROPICAL MEDICINE AND HYGIENE

Vol. 5 *November 1956* *No.* 6

CONTENTS

REVIEW ARTICLE

World-wide malaria distribution, prevalence, and control. PAUL F. RUSSELL . . 937

The time required to reach eradication in relation to malaria constitution. ARNOLDO GABALDON 966

A pilot project of residual-insecticide spraying to control malaria transmitted by the *Anopheles punctulatus* group in Netherlands New Guinea. D. METSELAAR 977

Some aspects of concomitant infections of plasmodia and schistosomes. I. The effect of *Schistosoma mansoni* on the course of infection of *Plasmodium berghei* in the field vole (*Microtus guentheri*). MEIR YOELI 988

Experimental *Entamoeba histolytica* infections in man. PAUL C. BEAVER, RODNEY C. JUNG, HARRY J. SHERMAN, THOMAS R. READ AND THOMAS A. ROBINSON 1000

Intestinal parasites in an Egyptian village of the Nile valley with emphasis on the protozoa. DEANER K. LAWLESS, ROBERT E. KUNTZ AND C. P. A. STROME 1010

Experimental chemoprophylaxis of amebiasis. PAUL C. BEAVER, RODNEY C. JUNG, HARRY J. SHERMAN, THOMAS R. READ AND THOMAS A. ROBINSON . . . 1015

Experimental transmission of yellow fever by Central American species of *Haemagogus* and *Sabethes chloropterus*. PEDRO GALINDO, ENID DE RODANICHE AND HAROLD TRAPIDO 1022

Studies of the Sindbis virus. Part I. Susceptibility of the cave bat by various routes of exposure. REGINALD L. REAGAN, FRANCES S. YANCEY, SING CHEN CHANG AND A. L. BRUECKNER 1032

Studies of the Sindbis virus. Part II. Susceptibility of the suckling hamster by various routes of exposure. REGINALD L. REAGAN, FRANCES S. YANCEY, SING CHEN CHANG AND A. L. BRUECKNER 1034

Some aspects of yaws in Liberia. F. S. DA CRUZ-FERREIRA AND H. STERENBERG 1036

The laboratory diagnosis of typhoid fever in areas of endemicity. K. C. WATSON AND W. LAURIE 1051

Studies of *Pasteurella pestis* in fleas. V. The experimental plague-vector efficiency of wild rodent fleas compared with *Xenopsylla cheopis*, together with observations on the influence of temperature. LEO KARTMAN AND FRANK M. PRINCE 1058

Some new comparative investigations on three *Physopsis* borne schistosomes: *Schistosoma haematobium*, *S. bovis* and *S. intercalatum*. J. SCHWETZ 1071

Studies on the helminth fauna of Alaska. XXX. The occurrence of *Echinococcus multilocularis* Leuckart, 1863, on the mainland of Alaska. ROBERT RAUSCH 1086

Mosquito collections and dissections for evaluating transmission of filariasis in Polynesia (Tahiti). DAVID D. BONNET, JOHN F. KESSEL, JACQUES KERREST AND HERALD CHAPMAN 1093

ACTH as an adjunct to the treatment of loaiasis. JAMES H. THOMPSON 1103

Books received 1106

Book reviews: *La Lèpre*, by Chaussinand; *The History of the School of Tropical Medicine in London*, by Manson-Bahr 1106

Index 1109

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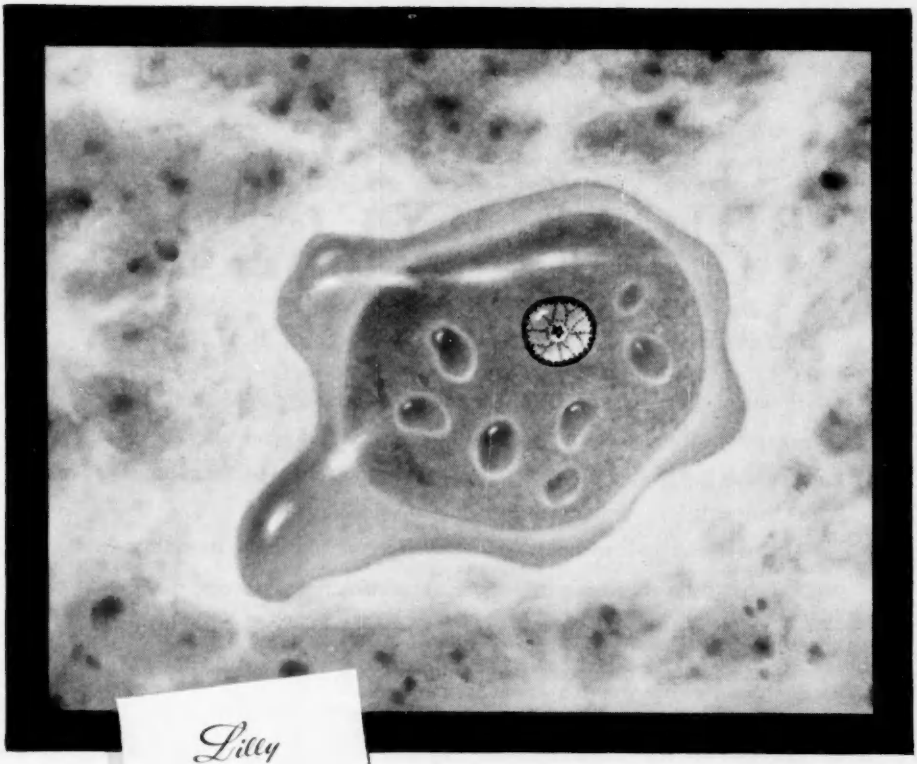

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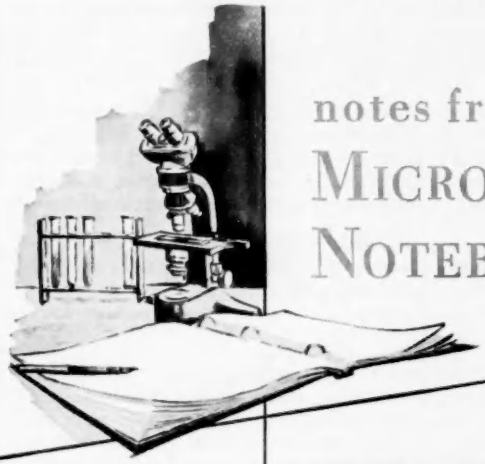

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The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

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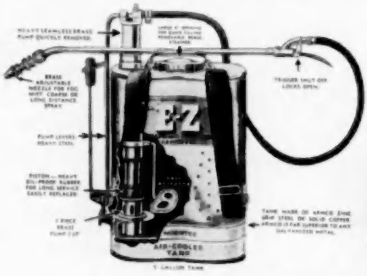

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WORLD-WIDE MALARIA DISTRIBUTION, PREVALENCE, AND CONTROL[1]

PAUL F. RUSSELL

The Rockefeller Foundation

Among all the plagues that afflict mankind, malaria has long been a leader. Today it is under an attack so widespread and determined that one can safely predict an early end to its supremacy. This paper discusses the world distribution and prevalence of malaria and the status of its control.

WORLD DISTRIBUTION

Very likely, malaria reached its maximum world coverage sometime between 1880, when it was endemic in southern Canada, and 1920 when it approached the Arctic Circle in Russia. Hirsch (1883) set the northern limit of malaria in the Americas at Kingston, Ontario, on the shore of Lake Ontario (48° 8′ N.) with occasional epidemics at Lake St. Peter on the St. Lawrence River (48° 10′ N.). Davidson (1892) stated that "Kingston and Toronto, situated on Lake Ontario, are undoubtedly malarious, although in a minor and diminishing degree." Hackett (1949) noted that during the unusually hot summers of 1920–21 malaria occurred on the Dvina River near Archangel (64° N.); also that each year between 1935 and 1938 more than a thousand fresh infections were reported from Krasnoborsk (61° 30′ N.). Since that pandemic, malaria has been retreating in Europe and also in America, notably during the past ten years, even dramatically in some areas.

At present the front lines of malaria are deployed somewhat as follows:

North America: The northern limit of malaria endemicity today may be said to be along the Rio Grande River at about 30° N. latitude.

South America: Malaria is not endemic in Uruguay and has only minimal prevalence in northeast Argentina. It still occurs in southeast Brazil but has been eradicated from Chile. Latitude 30° 20′ S. is approximately the southern limit.

Europe: Malaria is mildly endemic in the Netherlands and Germany, at approximately 52° 30′ N. Whether or not malaria now occurs in the USSR north of this latitude, I do not know.

Asia: Paucity of data makes it difficult to determine the northern limit of

[1] Malaria data in this paper refer to 1955 and often do not take note of considerable advances in 1956. It must also be emphasized that most malaria statistics are not accurate. Estimates from official sources vary remarkably for the same areas and time periods. Consequently, the figures given in this paper are to be taken as *indices* of the malaria situation in each country. The author made sustained efforts to obtain through personal visits, personal communications, a search of reports published and unpublished, and conferences with officials of governments and of WHO, UNICEF, and ICA, the most reliable estimates possible for each country. He believes that although the figures themselves are seldom strictly accurate, they do in fact give a reliable indication of the malaria situation as it was at the end of 1955.

malaria in Asia. But in the past it has been endemic in Irkutsk on Lake Baykal, at approximately 52° 30′ N.

Africa: The southern limit of malaria endemicity is probably about 29° 30′ S. on the Umvoti River, just south of Stanger, in eastern Natal. Malaria is endemic in the Canary, Cape Verde, Madagascar, Comoro, Réunion, and Mauritius Islands. The Medical Department of the Seychelles has reported that there are no anophelines and no malaria in the islands.

Australia: Malaria is only mildly if at all endemic in northern Australia at the present time. Certainly, it does not occur south of 20° S.

Oceania: In the central and south Pacific region, such islands as the Galapagos, Marquesas, Fiji, Samoa, Tonga, Cook, Hawaiian, New Caledonia, New Zealand, Marshalls, Marianas, and Carolinas are entirely without *Anopheles* and so are free of malaria. On Guam, *Anopheles subpictus indefinitus* appeared during World War II but no malaria transmission has occurred there. Most other islands of Oceania are highly malarious.

Malaria has occurred as high as 2770 meters (9086 feet) in the Cochabamba region of Bolivia, at 2591 m. (8500 ft.) near Londiani in Kenya, and at 2850 m. (9348 ft.) in Tadzhik SSR, south central Asia. It is still endemic in the Dead Sea basin at 400 m. (1312 ft.) below sea level.

WORLD PREVALENCE

As to the prevalence of malaria, the reliability of data is often so poor that one must take into account personal acquaintance and collateral evidence as well as formal reports. In the world's total population of some 2.653 billions, I estimate that, including communist China and the countries behind the Iron Curtain, probably no fewer than 1,070.280 millions live in areas where malaria is still, or has been within recent years, endemic. (See Table No. 1).

According to reports and estimates it seems that, throughout the world in 1955, some 375 millions were being routinely protected to some degree from malaria. (See Tables 1, 2, 5–14, 16–18.) This control varied from minimal to complete. How many cases of malaria occurred in 1955 among the some 695 millions living in unprotected or poorly protected endemic areas, we can only guess. We know that in the 1930's, when India's population was about 350 millions and malaria control was not common, competent observers like Sinton were estimating that some 100 million cases of malaria occurred each year, resulting in a million deaths. This was an incidence rate of about 29 per cent. All degrees of endemicity were known, under a great variety of climatic conditions. If we use this percentage figure as a basis, we may estimate that nearly 200 million cases of malaria occurred among the 695 millions exposed. Allowing for cases in so-called protected areas, it seems not unreasonable to estimate that in 1955 there were from 200 to 225 millions of cases of malaria in the world, with more than 2 million deaths due to the disease. Previous estimates have ranged from 250 to 350 million cases (Russell, 1952; Pampana and Russell, 1955).

TABLE 1

Malaria estimates: world summary

Locality	Population	Population in Malarious Areas	Population Routinely Protected	Population Poorly or Not at all Protected
North America	216.739	79.779	62.061	17.718
West Indies	17.537	8.491	5.266	3.225
South America	121.018	51.698	27.369	24.329
Totals, Americas	355.294	139.968	94.696	45.272
Northern and Northeastern Africa	73.347	35.308	14.536	20.772
Central and Western Africa	84.251	82.513	7.726	74.787
Southern Africa	32.843	21.445	6.893	14.552
Eastern Africa	20.375	17.862	.780	17.082
Totals, Africa	210.816	157.128	29.935	127.193
Totals, Europe	404.829	41.991	34.666	7.325
Near East	57.257	37.908	20.257	17.651
Arabian Area	13.272	9.840	1.122	8.718
Middle Asia	533.239	373.097	125.142	247.955
Far Eastern Asia	849.582	258.629	31.598	227.031
Totals, Asia	1,453.350	679.474	178.119	501.355
Totals, Oceania	14.268	1.719	.097	1.622
Totals, USSR	214.500	50.000	37.000	13.000
Totals, World	2,653.057	1,070.280	374.513	695.767

Notes: 1. Figures in millions.
2. Data summarized from Tables 2, 5–14, 16–18.

REGIONAL DISTRIBUTION, PREVALENCE, AND CONTROL

North America (Table No. 2)

Canada: malaria is no longer endemic in Canada although the vector *quadrimaculatus* is found in the southern fringes of Quebec and in Ontario from the Ottawa Valley southwards to Lake Erie. *Freeborni*, another vector, is present in small numbers in British Columbia.

United States: malaria has almost completely ceased to be endemic in the continental United States, although the former vectors, *quadrimaculatus* in the southeast and in the Mississippi Valley, and *freeborni* in California, are still prevalent. During 1954, there were only 8 confirmed primary indigenous cases and in 1955, only 4 (provisional figure). This is in sharp contrast to the findings

of von Ezdorf (1915, 1916) some 40 years ago, when 11 southern states reported a total of more than 780,000 cases of malaria. Von Ezdorf estimated that malaria deaths varied from one in each 50 cases in highly endemic areas, to one in every 300 cases where endemicity was mild. The following tables, Nos. 3 and 4, display data for the years 1948–1955. (Courtesy CDC, USPHS.)

TABLE 2

Malaria estimates: North America

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Alaska	208	0	—
Bermuda	40	0	—
British Honduras	77	75	40
Canada	15,195	0	—
Canal Zone	54	54	54
Costa Rica	915	350	265
El Salvador	2,122	1,282	1,282
Greenland	25	0	—
Guatemala	3,149	1,374	557
Honduras	1,608	1,203	316
Mexico	28,849	16,000	836
Nicaragua	1,202	1,000	670
Panama	886	650	250
U.S.A.	162,409	57,791	57,791
Totals	216,739	79,779	62,061

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 mid-year, unless otherwise noted.
3. Malaria data from Alvarado (1954) and others.

TABLE 3

Malaria morbidity reported and cases appraised in the United States, 1948–1955

Year	Number of Cases Reported (a)	Cases Appraised		
		Number	Fully confirmed as malaria	
			Total	Primary indigenous malaria
1948	9,797	770	242	—
1949	4,231	514	60	17
1950	2,227	713	30	6
1951	5,600	1,874 (c)	1,272	14
1952	7,023	3,098 (c)	2,707	33
1953	1,310	449	217	29
1954	715	130	97	8
1955	477 (b)	64 (b)	29 (b)	4 (b)

(a) Source-National Office of Vital Statistics, USPHS.
(b) Provisional only.
(c) Majority of cases were US Army Korean veterans.

TABLE 4

Location of confirmed primary indigenous cases of malaria in United States, 1953–1955. (Courtesy CDC-USPHS.)

Year	State	Cases	Comments
1953	California	24	Camp Fire Girls. Source–Korean veteran
	California	1	Source unknown
	Georgia	1	Source—Georgia
	Maryland	1	Source—Maryland
	Mississippi	1	Source—Mississippi
	S. Carolina	1	Source—Unknown
1954	Arizona	4	Children of one family. Source Korean veteran
	Arizona	1	Not associated with preceding
	S. Carolina	1	Source—Alabama?
	Mississippi	1	Source—Mississippi or Alabama
	Mississippi	1	Source—blood transfusion
1955	California	2	Separate in time and place. Source?
	Arizona	1	Source?
	Illinois	1	Source—blood transfusion

Mexico: the most extensive and severe malaria problem of the Americas is found in Mexico where 836 thousands of the estimated 16,000 thousands living in endemic areas have been under routine protection. Average annual malaria death rates in excess of 200 per 100,000 population have been noted in some States. Over 22,000 malaria deaths were reported in 1953. The vectors are *albimanus* in the coastal lowlands and in the valleys of large rivers; *darlingi* suspected in Yucatan; *aztecus* transmitting on the tableland; *pseudopunctipennis* at low and moderate elevations under semiarid and subtropical conditions. Roughly two-thirds of Mexican malaria is due to *P. vivax* and most of the rest to *P. falciparum*. A nation-wide malaria eradication project was inaugurated in Mexico in 1955 that will eventually involve the residual spraying of some 3.098 million homes and the expenditure of some $6.773 millions, in the years of maximum endeavor.

Guatemala: some 557 thousands of the 1,374 thousands living in malarious areas of Guatemala are under routine protective DDT spraying. The disease is endemic in all regions of the country, transmitted chiefly by *albimanus* and *pseudopunctipennis*, with some assistance from *darlingi*.

British Honduras: malaria has been a serious problem in British Honduras but is now under strong attack and is decreasing rapidly in incidence. More than half of those living in malarious areas are under routine protection. *Albimanus* has been the chief vector, with assistance from *darlingi*.

Honduras: in 1942–43, malaria was first among causes of death in Honduras and the disease has been widely and severely prevalent. But routine DDT house-spraying of more than 50,000 homes has sharply reduced malaria morbidity. *Albimanus* has been the chief vector.

El Salvador: all of the 1,282 thousands living in malarious areas of El Salvador are under routine protection by DDT spraying. The disease, formerly widespread,

has been sharply curbed. The vectors have been *albimanus* and *pseudopunctipennis*. A country-wide reconnaissance in 1940 indicated a fairly even division in the relative prevalences of *P. vivax*, *malariae*, and *falciparum*.

Nicaragua: more than half of those exposed to malaria in Nicaragua are under some DDT residual-spraying protection. The vectors are *albimanus* and *pseudopunctipennis*.

Costa Rica: malaria has been widely endemic in Costa Rica and it has sometimes been the second most important cause of death. A DDT residual spraying project was started in 1946 and now covers some 75 per cent of all malarious communities. There has been a sharp drop in incidence; for instance, malaria cases treated dropped from 3,222 in 1942, to 283 in 1952. *Albimanus* has been the chief vector. A country-wide reconnaissance in 1939 indicated that *P. vivax*, *malariae*, and *falciparum* are about equal in prevalence in the country.

Panama: malaria is now uncommon in the Canal Zone and in the urban areas of the Republic but is still prevalent in rural areas. However, active DDT residual spraying has greatly reduced the incidence. The program covers 44,000 houses, protecting some 250 thousands. *Albimanus* and *punctimacula* have been the chief vectors (Dehne, 1955)

West Indies (Table No. 5)

Bahamas: the Bahamas (and Bermuda) have always been relatively exempt from malaria although imported cases have not been uncommon.

Barbados: malaria is not endemic now in the Barbados.

Cuba: malaria has not been a major problem in Cuba, although yearly epidemics have occurred in localized areas. The disease still persists in some areas involving a population of some 650 thousands and controlled to some extent by drainage and larviciding. Rapid eradication seems possible if, as planned, residual DDT spraying is applied to some 120,000 homes. *Albimanus* has been the chief vector, possibly assisted at times by *crucians* and *vestitipennis*. Infections have been nearly evenly divided between *P. vivax* and *falciparum*.

Dominican Republic: malaria is no longer a serious problem in the Dominican Republic where some 160 thousand homes are routinely sprayed with residual DDT, protecting half the population at risk. Eradication should be achieved in the next few years. *Albimanus* has been the vector and *falciparum* malaria has predominated. In some areas *P. malariae* has constituted 10 per cent of the infections.

Guadaloupe: malaria in Guadaloupe is transmitted by *aquasalis*, *albimanus*, and *argyritarsis*. Some 200,000 live in endemic areas and the disease has been a serious problem. A control project was started in 1950 that included not only DDT and BHC house-spraying but also larviciding in water-courses and marshes, and some active chemoprophylaxis, which in 1952 was applied to 24,257 children. Results have been excellent, worker output has improved, and absenteeism has decreased (Sautet and Aldighieri, 1954).

Haiti: there is still a large malaria problem in Haiti, where some 1,007 thousands of the 1,700 thousands at risk are being protected with residual DDT

TABLE 5

Malaria estimates: West Indies

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Bahamas	90	0	—
Barbados	225	0	—
Cuba	5,807 (a)	650	50
Dominican Republic	2,347	2,200	1,100
Guadaloupe	229	200	78
Haiti	3,506	1,700	1,007
Jamaica (b)	1,533	1,250	910
Leeward Islands (c)	124	20	20
Martinique	239	230	100
Netherlands Antilles	184	0	—
Puerto Rico	2,229	1,280	1,280
St. Pierre and Miquelon	5	0	—
Trinidad and Tobago	698	698	592
Virgin Islands (d)	24	0	—
Windward Islands (e)	297	263	129
Totals	17,537	8,491	5,266

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 mid-year, unless otherwise noted.
3. Malaria data from Alvarado (1954) and others.
4. (a) 1953; (b) including Cayman Islands, Turks and Caicos Islands; (c) including Antigua, Montserrat, St. Kitts-Nevis and Anguilla, and Virgin Islands (U.K.); (d) U.S.A.; (e) including Dominica, Grenada, St. Lucia, and St. Vincent.

spraying. Financial considerations have delayed expansion of the control program. *Albimanus* is the chief vector. Parasite indices have been as high as 50 per cent, with *P. falciparum* predominating.

Jamaica: malaria is still endemic, but mildly so, on Jamaica where nearly all who live in endemic areas are under routine protection. *Falciparum* and quartan malaria have predominated but *vivax* infections have been uncommon. *Albimanus* is the most common anopheline and the chief vector.

Windward Islands (Grenada, St. Vincent, St. Lucia, and Dominica) and *Leeward Islands* (Antigua, Montserrat, Anguilla, and St. Kitts-Nevis): In these two groups of islands, the chief vector is *aquasalis*, possibly assisted in the Leeward Group by *albimanus*. *Plasmodium falciparum* predominates but *malariae* is common while *vivax* is not. Grenada, St. Lucia, Dominica, and Antigua have been the most malarious, with parasite rates of 8.7, 12.9, 10.4, and 7.8 per cent, respectively. St. Kitts-Nevis, and St. Vincent, have been only mildly malarious, with parasite rates of 1.9, 0.0, and 2.2 per cent, respectively. Montserrat, normally not malarious, experienced an epidemic in 1938–39 (Charles, 1952). Good control with residual spraying is routine. Malaria eradication is almost complete in Antiqua and should be possible throughout the Islands within a few years.

Martinique: there have been serious endemic foci of malaria in Martinique but a residual spraying project including some 24,000 homes is reducing the malaria problem. *Albimanus* is not found in Martinique where the vector is *aquasalis*.

Netherlands Antilles: malaria is not endemic in the Netherlands Antilles.

Puerto Rico: malaria in 1931 was responsible for 3,208 deaths and 47,656 cases, in a population of 1.58 millions but now has been all but eradicated from Puerto Rico. The vector was *albimanus*. In 1954, only 13 cases were reported, 5 in Korean veterans and 3 in sailors in transit. No primary indigenous case was found. Control has been based on residual DDT, case finding, and treatment, aided by some major drainage during World War II.

Trinidad and Tobago: malaria incidence in the Colony of Trinidad and Tobago has sharply declined in the past 10 years under a strong attack that has included DDT residual spraying against *aquasalis* and *albitarsis*, and the spraying of copper sulphate solution on bromeliads on Trinidad to control *bellator* (*neomaculipalpis* may be a minor vector). There was an interesting change in the ratio of *falciparum* to *vivax* on Trinidad from 80.7/18.2 in 1952, to 61.75/36.5 in 1953. The percentage of parasite-positive slides declined from 19.4 to 11.5 per cent. On Tobago, where *falciparum* malaria predominates, and where *aquasalis* is the vector, spleen rates that exceeded 20 per cent in 1941 have fallen to nil in many cases. Residual spraying, larviciding, permanent elimination of major breeding places, antimalaria treatment have all but ended malaria transmission. *Albimanus* does not occur on Trinidad or Tobago.

Virgin Islands: malaria is not endemic in the Virgin Islands.

South America (Table No. 6)

Colombia: the malaria problem in Colombia is the second largest of the Americas, but some 3,100 thousands of the 7 millions at risk are now under more or less routine protection. In the Llanos, *darlingi*-transmitted malaria has long been severe, with natural fluctuations due to periodic waxing and waning of the vector. Residual spraying has reduced spleen and parasite indices in some areas to single figures within a four-year period. *Albimanus, albitarsis, pseudopunctipennis,* and *punctimacula* all have had a part in the transmission of malaria in Colombia.

Venezuela: malaria was the chief public health problem in Venezuela until recently. It was the chief cause of death annually from 1905 until 1945. But this country has led the way in planning nation-wide malaria eradication and the entire population at risk is now under routine protection. A well-organized Malaria Institute has been the power house of the project. This Institute has also trained a majority of the professional malariologists of Latin America. Great progress has been made towards eradication since the nation-wide project started in 1945. In one large north-central area, where severe and often epidemic malaria, predominately *falciparum*, was transmitted by *darlingi, albimanus*, and *albitarsis*, the disease has now been eradicated. Careful and prolonged surveys have checked this area (Gabaldon and Berti, 1954).

British Guiana: the only vector in British Guiana has been *darlingi* which now seems to have been eradicated from the coastal areas by means of residual DDT

TABLE 6

Malaria estimates: South America

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Argentina	18,742	1,855	1,855
Bolivia	3,162	1,300	411
Brazil	57,098	30,893	13,650
British Guiana	497	430	400
Chile	6,447	0	—
Colombia	12,382	7,000	3,100
Ecuador	3,567	1,420	887
Falkland Islands	2	0	—
French Guiana	28	28	28
Paraguay	1,530	510	25
Peru	9,213	3,773	2,544
Surinam	220	200	180
Uruguay	2,525 (a)	0	—
Venezuela	5,605	4,289	4,289
Totals	121,018	51,698	27,369

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 mid-year, unless otherwise noted.
3. Malaria data from Alvarado (1954) and others.
4. (a) 1953.

house-spraying. Consequently, malaria has been all but eradicated in the Counties of Demerara, Beabice, and Essequibo, where 95 per cent of the country's population resides. Small numbers of *darlingi* reappeared in 1951 near a new drainage and irrigation scheme. The reinvasion was quickly stopped with DDT residual spraying but it illustrated the fact that so-called "end points" are deceptive and that sustained surveillance for a number of years is essential before eradication can safely be proclaimed (Giglioli, 1954).

Surinam: malaria is still a problem in Surinam. *Aquasalis* is a probable vector in coastal areas and *darlingi* in the interior, along the savannah belt and near the sea in the extreme east. In coastal regions, *vivax* malaria predominates in some areas and *malariae* in others. Severe *falciparum* malaria is prevalent in the interior. The DDT spraying project does not yet cover half the population at risk.

French Guiana: malaria was severely endemic in French Guiana prior to 1949, affecting the entire coastal population and forested interior. The annual cost of hospitalization for malaria was about a million francs and the disease was responsible for economic stagnation. A DDT residual spraying project was started in 1949 and has been dramatically successful. Malaria hospitalization costs in 1953, for example, had fallen to the low figure of some 65,000 francs. There has been a 98.9 per cent reduction in positive blood smears, and the economic stagnation has vanished. The total costs of the program up to 1954 had been some 8.1 millions of francs and the savings in hospital care and wages earned had been estimated for 1951 alone as some 25 million francs (Floch, 1954).

Brazil: DDT residual spraying has had good success in Brazil against the chief vectors, *darlingi*, *tarsimaculatus*, *albitarsis*, *cruzii*, and *bellator*. An over-all decrease of 95 per cent in malaria incidence has been estimated as due to this spraying program. In coastal regions, malaria eradication has followed two sprayings a year for three years. However, in some areas of the central plateau, *darlingi* attacks man in the open and, moreover, in the west many inhabitants live in wall-less shelters. These conditions and in some areas, the out-door resting and feeding habits of *bellator* and other Kertészia species greatly reduce the effectiveness of residual spray. Another problem is found in the western part of the Amazon region where a scattered, primitive population is relatively inaccessible. Here chloroquine has been added to the Government table-salt, the only salt available to the people. This measure has had some initial success (Pinotti, 1954, 1955).

Ecuador: malaria is prevalent throughout the coastal provinces of Ecuador and in many of the interior valleys. *Albimanus* is the chief vector in low-lying coastal areas, assisted by *pseudopunctipennis* and *punctimacula*. In the valleys of the Andes Provinces, *pseudopunctipennis* is the chief and perennial vector. A national antimalaria campaign was started in 1946 and as a result of DDT residual spraying twice a year, malaria incidence and severity have declined considerably. More than 60 per cent of those living in malarious areas are under routine protection. In the low-lying provinces, *P. falciparum* predominates, but *vivax* is most prevalent in the Andean areas, with *malariae* fairly common (Montalván, 1952).

Peru: there is still a severe malaria problem in Peru where the vectors are *pseudopunctipennis*, *albimanus*, and *punctimacula*. Some 2,544 thousands of the 3,773 thousands estimated to be at risk are now under more or less routine protection by residual spraying and in some areas incidence and intensity of the disease have sharply declined.

Bolivia: malaria has long been severe in all but the highest areas of Bolivia. The chief vectors have been *darlingi* and *pseudopunctipennis*. Some 411 thousands of the 1,300 thousands at risk are now under routine DDT spraying protection and malaria indices have sharply fallen.

Paraguay: malaria data from Paraguay are insufficient to make the malaria situation clear but it is estimated that some 510 thousands are at risk and that of these some 25 thousands have had a certain amount of protection. Malaria vectors have not been definitely incriminated but it seems likely that *albitarsis* and *darlingi* are involved. Spleen indices above 50 per cent have been reported.

Chile: malaria used to be endemic in northern Chile, transmitted by *pseudopunctipennis*. An antimalaria campaign was begun in July, 1937, and by 1941 covered the entire endemic area. The advent of DDT helped to consolidate the complete success of the project. Not a single autochthonous case of malaria has been diagnosed in Chile since April, 1945 (Neghme *et al.*, 1955). No adults of the former vector species have been caught since the fall of 1948 but there have been and may still be a few foci of larvae near the Peruvian border.

Argentina: malaria was formerly endemic in northeastern and northwestern Argentina. It has now been eradicated from the Provinces of La Rioja, San Luis, Cordoba, San Juan, and Catamarca, and all but eradicated from the rest of the

area. *Pseudopunctipennis* has been the vector and *P. vivax* the predominating parasite.

Uruguay: malaria is not endemic in Uruguay.

Europe (Table No. 7)

Malaria has sharply retreated in Europe during the past 75 years. Hardly a country on the continent was free of endemic malaria in the 19th Century. Sweden, for instance, prior to 1880, often had 5,000 or more cases a year and the disease afflicted Finland and the Baltic States. World Wars I and II resulted in some resurgence of the intermittent fevers in Scandinavia but at present they are rarely seen in Western Europe north of The Netherlands. France and Belgium were highly malarious in some areas before 1875 but only sporadic cases have occurred in the past few years. The marshy region between the estuaries Charent and Seudre on the west coast of France was heavily infested with intermittent fevers during the 17th, 18th, and 19th centuries. Rochefort suffered severely. In a recent period of 15 years some 34 locally contracted *vivax* infections were noted and the vector, *atroparvus*, is still common (Bonnin *et al.*, 1952). The disease remains endemic in Corsica where *labranchiae* is the chief vector, but the incidence has been much reduced in recent years. Switzerland was malarious in certain valleys 75 or 80 years ago but is now free of the disease. Marsh areas of the British Isles, even into Scotland (but never in Ireland), were once quite malarious. There were brief and circumscribed malaria episodes in some of the old foci in England after the World Wars but it is doubtful if the disease can now be called endemic in the United Kingdom. However, between 1917 and 1952, some 566 cases of indigenous malaria were recorded in the British Isles, all but one due to *P. vivax*. The chief vector appears to have been *atroparvus* but no sporozoites have been found in wild-caught specimens. Tree-hole breeding *plumbeus* (or perhaps *claviger*, breeding in tanks) may have transmitted *falciparum* malaria in one case in Liverpool in 1920 and *vivax* malaria in two cases in London in 1953 (Shute, 1954).

Only a single focus of malaria now exists in Western Europe north of the Alps. This is an area of *atroparvus*-transmitted malaria between Amsterdam and Wilhelmshaven in The Netherlands provinces of North Holland, Friesland, Zeeland, and in German East Friesland. North Holland reported 26 autochthonous cases in 1953, 15 in 1954, and 11 in 1955 (Swellengrebel, 1955, 1956, personal communication). No locally contracted cases were reported elsewhere in The Netherlands in 1953 and 1954. Following World War II, more than 100 autochthonous cases of malaria were noted in German northern Westphalia but this focus appears to have faded out.

Portugal and Spain still have endemic, *atroparvus*-transmitted malaria and there is a localized persistent focus of *labranchiae* malaria in a small area of southeast Spain. In Portugal, *vivax* malaria predominates in the north but, frequently, *falciparum* in the south. Over the entire Iberian Peninsula malaria has retreated in response to recent control measures. Spain reported 6,624 cases of malaria in 1953 and 4,340 in 1954 with no malaria deaths (Clavero, 1955, 1956, personal communication). Portugal reported 15,200 positive cases in 1948, 608 in

TABLE 7

Malaria estimates: Europe

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Albania	1,260	1,000 (e)	500 (e)
Andorra	5	0	—
Austria	6,969	0	—
Belgium	8,819	0	—
Bulgaria	7,500 (a)	2,000	2,000
Channel Islands	103	0	—
Czechoslovakia	12,952	1,000	1,000
Denmark (b)	4,439	0	—
Finland	4,190	0	—
France (c)	43,000	145 (d)	145 (d)
Germany	70,000	1,000	1,000
Gibraltar	25	0	—
Greece	7,901	4,500	4,500
Hungary	9,691	1,500 (e)	1,000 (e)
Iceland	154	0	—
Ireland	2,933	0	—
Italy	47,665	10,000	10,000
Liechtenstein	14	0	—
Luxembourg	306	0	—
Malta and Gozo	320	0	—
Monaco	22	0	—
Netherlands	10,615	2,696	2,696
Norway	3,392	0	—
Poland	26,500 (a)	1,000 (e)	500 (e)
Portugal	8,693	950	375
Roumania	17,150	3,000	3,000 (e)
Saar	983	0	—
San Marino	13	0	—
Spain	28,751	8,000	2,750
Sweden	7,214	0	—
Switzerland	4,923	0	—
United Kingdom	51,059	0	—
Vatican City	1	0	—
Yugoslavia	17,267	5,200	5,200
Totals	404,829	41,991	34,666

Notes: 1. Figures in thousands.

2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 mid-year, unless otherwise noted.

3. Malaria data from WHO, from personal communications and, as noted, by author's estimates.

4. (a) 1953; (b) including Faeroe Islands; (c) excluding North Africa. See table No. 11; (d) Malaria data refer to Corsica; (e) Author's estimate.

1953, 409 in 1954, and 184 in 1955 (Roque, 1955, personal communication). The disease is still endemic in Spain's Canary Islands but the Balearic Islands, once severely infested, are now nearly free of the disease.

Malaria remains endemic in Poland, Czechoslovakia, Hungary, Bulgaria, Roumania, Jugoslavia, Albania, and the USSR. Data are scarce from these countries but observers have noted that malaria is under attack in each country with some house-spraying and with considerable reliance on compulsory case-reporting, clinical and suppressive treatment. In these countries, it seems likely that *messeae* is the chief vector. In Albania, *sacharovi* and *superpictus* are important and this is also true to some extent in Bulgaria and Roumania.

In Albania, prior to 1933, some 500,000 cases of malaria occurred annually. Spleen rates often exceeded 50 per cent. Residual spraying with DDT has been employed since 1947 and considerable use is made of prophylactic chlorguanide. Spleen rates have fallen sharply in several areas. Some DDT larviciding is done in urban and suburban areas.

Bulgaria also used to have half a million or more cases of malaria yearly but has been using DDT residual spraying since 1949–50 and has enforced compulsory malaria therapy as a prime measure of control. All cases are supposed to receive therapeutic doses of quinine, quinacrine, or Plasmocid (a Russian product resembling plasmochin and probably identical with Rhodoquine). This therapy is followed by two years' prophylactic treatment with Quinoplasmine (a quinine-plasmochin combination). Great reduction in malaria incidence is claimed.

In 1948, Roumania reported some 338,000 cases of malaria. Since then incidence has steadily fallen and in 1954 only 916 cases were recorded (Pampana 1955, 1956, personal communication). All residents of endemic areas are under routine protection by a combination of residual DDT or BHC spraying, therapeutic measures, and mass drug prophylaxis. Quinacrine, chlorguanide, or chloroquine are used therapeutically and collective use of prophylactic quinacrine or chlorguanide is standard practice in all communities exposed to infection.

The USSR (Table No. 8) has been highly malarious in the past in the great river basins extending from the Black and Caspian Seas almost to the Arctic Ocean. In Azerbaidzhan, *maculipennis, superpictus,* and *claviger (bifurcatus)* are vectors, chiefly of *vivax* malaria. Elsewhere in the USSR the chief vector is *messeae*. Indirectly, we know that considerable progress has been made in malaria control through compulsory reporting and treating of cases, "collective drug prophylaxis," some house-spraying with BHC or DDT, and some larva control

TABLE 8

Malaria estimates: USSR

Locality	Population	Population in Malarious Areas	Population Routinely Protected
USSR—Europe and Asia	214,500	50,000	37,000

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955.
3. Malaria data are author's estimates, in thousands.

with drainage and larvicides. Residual spraying is sometimes "nidal" or "focal," applied only to houses of malaria patients; sometimes "barrier," applied only to houses nearest a swampy area; and sometimes "blanket," applied to all structures in a community. In some areas, cattle have been sprayed with DDT as a measure of malaria control.

Great strides towards full control of malaria have been made in Yugoslavia, formerly highly malarious in some areas. There were only 992 microscopically diagnosed cases in 1953 and 2,155 in 1954, the increase being largely in Macedonia (Djordjevic, 1955, personal communication). This is in striking contrast to the several hundred thousand cases formerly reported annually.

Italy has practically eradicated malaria. Only 9 primary cases were reported in 1953, 6 in 1954, and in 1955 only 5, of which 2 were quartan malaria following transfusions (Raffaele, 1956, personal communication). These reports covered not only the mainland but also Sicily and Sardinia.

Greece was once one of the most malarious countries outside the Tropics. In 1932 there were 7,042 deaths from malaria with 1,408,400 cases and in 1942 the death toil was 1,856. The incidence of the disease has been greatly reduced by residual spraying. Parasite indices in children averaged 17.2 per cent, 1933–1945 but had fallen to 0.09 per cent in 1954. Laboratory-proven cases totalled 399 in 1952, 1,403 in 1953, 2,350 in 1954 and 1,624 in 1955, the increase after 1952 being due to development of DDT resistance by *sacharovi* (*elutus*). The total number of cases estimated for 1953, and again in 1954, were 12,000 to 15,000. Two malaria deaths each year in 1953 and 1954 were reported from towns of over 5,000 population. In 1955 no deaths from towns of 10,000 or more were reported up to the end of August (Belios, 1955, 1956, personal communication). The vectors are *sacharovi* along the coast, *maculipennis* in the valleys and plains, and *superpictus* along stream beds.

Near East (Table No. 9)

Cyprus: some 500,000 of the 514,000 inhabitants of Cyprus live in areas formerly malarious. In 1937, 18,273 cases were reported and in 1945, 7,686 cases. But the disease was eradicated during a special project started in 1946 and ended in 1950. No autochthonous case has been reported since 1951. Annual parasite indices have been nil since 1953. The chief vectors were *superpictus*, *sacharovi* and *claviger*. *Sacharovi* appears to have been eradicated. No adult *superpictus* have been taken since 1953 but small numbers of larvae are still found.

Turkey: in times past Turkey has suffered greatly from *sacharovi* and *superpictus* malaria but an extensive control program in operation since 1925 has greatly reduced the incidence in some 14,000 of 30,000 villages where the disease is endemic. Yearly incidence of malaria in the hospitals of the villages under control has declined from some 17,000 in 1942 and again in 1943, to 4,887 in 1953 and 4,229 in 1954. Malaria deaths recorded were 600 in 1942 and 818 in 1943 but had dropped to 19 in 1953 and 21 in 1954 (Pek, 1955, personal communication).

Lebanon: malaria is under good control and should soon be eradicated in 750 villages of Lebanon in which the disease is endemic. The vectors have been

TABLE 9

Malaria estimates: Near East

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Cyprus	514	500	500
Iran	20,721	12,000	3,885
Iraq	4,948	3,000	2,900
Israel	1,688	1,773	1,630
Jordan	1,384	1,185	200
Lebanon	1,383	300	262
Syria	3,670	1,150	240
Turkey	22,949	18,000	10,640
Totals	57,257	37,908	20,257

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from personal communications and WHO.

sacharovi and *superpictus* perhaps associated with *claviger* in some areas. *Sergenti*, of importance in Jordan and Israel, has not been proved to be a vector in Lebanon. Prior to 1946 spleen indices were frequently between 70 and 90 per cent. In over 9,000 blood films examined in 1955, only 30 were positive—one *falciparum* and the others *vivax*.

Syria: malaria is still common in Syria but an eradication scheme is now being organized. The vectors are *sacharovi*, *superpictus*, and in the south, *sergenti*. The large cities of Damascus and Aleppo are not malarious but in the rural areas some 1,150 thousands of the 3.67 million population live in malarious areas.

Jordan: some 1,185 thousands of Jordan's 1.38 millions population live in malarious areas and the disease is one of the most important causes of morbidity. Control measures are being organized. *Sergenti* presents a real problem in the Yarmouk and Jordan valleys because it seldom enters houses but prefers caves and thus is not amenable to residual spraying. It transmits in October and November principally. *Sacharovi* is the spring and summer vector, *superpictus* transmits chiefly between July and September. *Claviger* is not a vector of any importance in Jordan.

Iraq: some 3 millions of Iraq's 5 millions live in malarious areas but the disease is coming under effective control. Areas under routine protective measures have a population of some 2.9 millions. The principal vectors in Iraq are *stephensi*, *sacharovi*, *superpictus*, and *maculipennis*. Formerly, both *stephensi* and *sacharovi* were active in the Shatt al-Arab and Hamman areas of south Iraq, but *sacharovi* has disappeared there since 1948, following control measures. Elsewhere throughout Iraq, *superpictus* is the chief vector, except above 3,000 feet where *maculipennis* alone transmits. *Sacharovi* is important in the alluvial plain and sub-montane communities of central and south-central Iraq. It is often an autumnal vector and in some areas transmits throughout the mild winters (Pringle, 1954, 1955).

Israel: malaria incidence is much reduced in Israel, the total incidence in 1955 being only 94 cases, (Mer, 1956, personal communication). Formerly, malaria occurred in two waves, with peaks each year in July and November. Now the disease has only one peak, coming in October. *Sacharovi* in the coastal zones, which was largely responsible for the summer epidemic, has nearly disappeared. *Superpictus*, the summer vector in hilly areas, has been greatly reduced in numbers. But *sergenti* has increased following development of irrigation and fish ponds. As in Jordan, so in Israel, *sergenti* is little affected by DDT residual spraying because it does not rest on treated surfaces, preferring to rest in caves, although it feeds both indoors and outdoors. September–October is the time of its greatest activity.

Iran: some 12 of Iran's 20.7 millions live in malarious areas and the disease is one of the country's chief health problems. The vectors are *maculipennis* in the northeast, north, northwest, and west; *sacharovi* in the north, northwest, and west; *culicifacies* in the southwest, south, southeast, east, and central; *stephensi* in the southwest, south, southeast, and central; *superpictus* here and there throughout the country. *Fluviatilis*, *multicolor*, and *pulcherrimus* are under suspicion in some communities. A malaria eradication project is being implemented and gives promise of success.

Arabian Area (Table No. 10)

Data are incomplete for the Arabian area so that only wide estimates can be offered as to malaria incidence. The anopheline fauna is African in the west, with *gambiae* an important vector, assisted by *sergenti* and *pretoriensis*. *Culicifacies*, *stephensi*, *sergenti*, and *fluviatilis* are vectors in the east.

Of the 7 millions resident in Saudi Arabia, some 5 millions are nomadic, the

TABLE 10

Malaria estimates: Arabian Area

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Aden and Protectorate	800	400 (b)	150
Bahrain Islands	112	110 (b)	100 (b)
Kuwait	200	0	—
Muscat and Oman	550	300 (b)	10 (b)
Qatar	30	0	—
Saudi Arabia	7,000	6,000 (b)	850 (b)
Trucial Oman	80	30 (b)	1 (b)
Yemen	4,500 (a)	3,000 (b)	11 (b)
Totals	13,272	9,840	1,122

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from personal communications and WHO.
4. (a) 1949; (b) author's estimate.

rest living in cities and towns. Some 6 millions of the total population are subject to the risk of malaria infection. The Aramco Health Section in Dharhan has carried out good malaria control since 1948 among some 250,000 population. On the west coast, a WHO team began a malaria control demonstration in 1952 and in 1955 did residual spraying in rural towns having a total population of some 100,000 and also did larviciding in Mecca and Gedda for the protection of some 500,000 population (Farid, 1956, personal communication).

Malaria has not been a serious problem in Aden and its Protectorate but there are occasional localized epidemics. The disease is still common in Muscat and Oman but is under good control in the Bahrain Islands. There appears to be no malaria in Kuwait and Qatar. Malaria is one of the principal causes of sickness and death along the Trucial Coast and in Yemen. The disease is most severe in the middle heights, next in the lowlands and least in the highlands. Among the anophelines there, only *gambiae* has been found infected but *adenensis* and *sergenti* are under suspicion (Mount, 1953).

Northern and Northeastern Africa (Table No. 11)

In the Moroccos, Algeria, and Tunisia, the chief vector is *labranchiae*, found in fresh and brackish waters of rivers and marshes. In desert oases *sergenti* is the usual vector, sometimes assisted by *multicolor* which is occasionally the only

TABLE 11

Malaria estimates: Northern and Northeastern Africa

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Algeria	9,369	3,500	1,407
British Somaliland	640	640	10
Egypt	22,651	12,000	8,000
Eritrea	1,000	500	50
Ethiopia	15,000 (a)	5,000 (b)	20 (b)
French Somaliland	63	63	35
Italian Somaliland	1,269	1,200	125
Libya	1,092	300 (b)	15 (b)
Morocco	8,340	2,600	1,936
Spanish Morocco	1,017	75 (b)	10 (b)
Spanish North Africa	143	20 (b)	1 (b)
Sudan	8,900	8,500	2,337
Tangier	183	0 (b)	—
Tunisia	3,680	910	590
Totals	73,347	35,308	14,536

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from personal communications and WHO and, as noted, by author's estimate.
4. (a) 1951; (b) author's estimate.

vector, as in the Algerian Quargle Oasis. Malaria is now prevalent and sometimes severe in the coastal belt but also occurs in the high valleys of the Atlas Mountains up to 8,000 feet, probably transmitted by *hispaniola*. Libya has less rainfall and less malaria. *Labranchiae*, present in the northwest is not found in eastern Libya where *sergenti* is the chief vector.

Malaria incidence has declined considerably since World War II in all of these countries. For instance, in Algeria the percentage of malaria cases diagnosed among all cases seen at clinics dropped from 14.33 in 1950 to 7.79 in 1954. Primary malaria cases reported in 1948 totalled 12,741 but were 4,413 in 1954 (Andarelli, 1955, personal communication). In Morocco, 246,171 cases of malaria were reported in 1948 but this figure had fallen to 49,085 in 1954. In every 1,000 consultations in 1948 there were 28.5 diagnoses of malaria but in 1954 only 2.6.

Egypt: malaria is still moderately endemic in Egypt but some 8 of the 12 millions who live in exposed areas are under routine protection. The devastating epidemic due to *gambiae* in 1942–1943 was exceptional. Since February, 1945, when the last *gambiae* was found in south Egypt following an intensive eradication project, this species has not been a source of malaria in the country. The principal oasis vector is *sergenti*, breeding in waste well-water and canals. A secondary vector in oases and the prime carrier elsewhere in Egypt is *pharoensis*, found in still rice-field and surface water. Adults of *pharoensis* are less given to house-haunting in the daytime than those of *sergenti* (the latter is not house-haunting in Jordan). *Multicolor* has often been suspected in Egypt but has not been definitely incriminated.

Sudan: of the 8.9 millions living in Sudan, some 8.5 millions are in areas where malaria is endemic and about 2.3 millions are under routine protection. Malaria remains a serious problem in some parts of this young nation.

Ethiopia: malaria is rare in Ethiopia above 6,500 feet and is not abundant in the 5,500–6,500 foot zone, although epidemics may occur. Below 5,000 feet the disease is widespread and often highly endemic. Spleen rates above 60 per cent are common. The principal season is that following the southwest monsoon, *i.e.*, from late September to mid-December. The disease is predominately epidemic in form except in the relatively few areas where perennial breeding places of the vector exist. Proximity to streams and river beds usually determines the degree of endemicity (Covell, 1955, personal communication). The principal vector appears to be *gambiae* with assistance from *funestus*, *pharoensis*, and *d'thali* in some areas. *P. falciparum* is the predominant parasite.

Africa South of the Sahara (Tables Nos. 12–14)

In most of Africa, south of the Sahara and north of the Union of South Africa, the climate is such that malaria vectors and parasites have assistance throughout the year and transmission is perennial wherever rainfall is well distributed. As latitude increases north and south and as wet zones become savannah and desert, transmission decreases. In general, malaria transmission is perennial along the East and West Coasts and in much of the center where altitude is below 1,400 meters and the latitude between 10° N. and 10° S. The cold Benguela current

TABLE 12

Malaria estimates: Central and Western Africa

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Angola	4,243	4,243	1,300
Belgian Congo	12,264	12,154	1,000
British Cameroons	1,460	1,441	100
British Togoland	423	416	10
Cape Verde Islands	166	111	86
French Cameroons	3,121 (b)	3,121	260
French Equatorial Africa	3,480 (a)	3,480	1,115
French Togoland	1,031 (b)	1,031	56
French West Africa	14,938 (a)	14,500	1,060
Gambia	273	273	20
Gold Coast	4,125	4,125	415
Liberia	1,250	1,250	150
Nigeria	30,300	30,300	1,542
Portuguese Guinea	535	535	10
Ruanda-Urundi	4,262	3,250	462
São Tomé and Principe	53	53	50
Sierre Leone	2,040	2,025	80
Spanish Guinea	205	205 (c)	10
Spanish West Africa	82	0 (c)	—
Totals	84,251	82,513	7,726

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from personal communications and from WHO; also, as noted, by author's estimate.
4. (a) 1937; (b) 1952; and, (c) author's estimate.

along the West Coast shortens the transmission season in some areas. From about 1,800 meters to 2,000 meters, malaria is more often epidemic in type.

As Macdonald has pointed out, the most notable aspects of the epidemiology of malaria in equatorial Africa are the intensity and the stability of the disease, due to a short extrinsic incubation period of the parasite and the highly susceptible, relatively long-lived anophelines that regularly feed on man. Malaria is highly endemic along the West Coast from latitude 8° N. southwards to about 10° S.; in the southern part of French Equatorial Africa and the Congo; the central Congo and northwest Angola; in the swampy northern Ovamboland and Okavango areas of South West Africa; in Uganda, Kenya, and Tanganyika below 1,400 meters; in Nyasaland and northern Mozambique below about 1,000 meters; along the East Coast to Natal; in the low veld of the Transvaal and in the bush veld area of Swaziland; in areas of Madagascar. Basutoland has never been malarious. Due to high altitude the climate is cold and malaria vectors are not found (DeMeillon, 1956, personal communication). Endemicity is intense in the greater part of the

TABLE 13

Malaria estimates: Southern Africa

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Basutoland	588	0	—
Bechuanaland	295	180	33
Comoro Islands	169	100	10
Federation of Rhodesia and Nyasaland	6,876	6,200	1,395
Madagascar	4,540 (a)	4,540	3,663
Mozambique	5,975	5,975	622
Réunion	274	230	230
St. Helena	5	0	—
Seychelles	37	0	—
Southwest Africa	447	100 (b)	20 (b)
Swaziland	212	120	120
Union of South Africa	13,425	4,000	800
Totals	32,843	21,445	6,893

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from WHO and personal communications, and, as noted, by author's estimate.
4. (a) 1953; and, (b) author's estimate.

TABLE 14

Malaria estimates: Eastern Africa

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Kenya	5,947	4,200	50
Mauritius	530	400	400
Tanganyika	8,196	8,000	230
Uganda	5,425	5,100	50
Zanzibar	277	162	50
Totals	20,375	17,862	780

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear.
3. Malaria data from WHO and personal communications.

western zone in North Nigeria, Dahomey, Ivory Coast, Gold Coast, French Guinea, and eastern Portuguese Guinea, and in the greater part of Rhodesia.

The Cape Verde Islands are highly malarious, *P. vivax* predominating, transmitted by *gambiae*. It is reported that this species has been eradicated from the island of Sol, the most northeasterly of the group, by DDT residual spraying. Malaria has been reported to be endemic in Teneriffe, Canary Islands, trans-

mitted probably by either *sergenti* or *hispaniola*, both of which are common (Galvan, 1952).

The three most important African vectors are *gambiae*, *gambiae melas*, and *funestus*. Judging by behavior characteristics, *gambiae* may be a complex of species although only *melas* has thus far been differentiated with certainty. Seven others, of the 30 Ethiopian anophelines, have local or secondary importance in malaria transmission, although they are greatly overshadowed by the big three. The seven are *A. brunnipes*, *hancocki*, *hargreavesi*, *moucheti*, *nili*, *pharoensis*, and *rufipes*.

Data regarding morbidity and mortality from malaria in Tropical Africa (see Tables 12–14) are thoroughly inadequate, which is not surprising in view of the inadequacy of medical personnel and facilities. Bruce-Chwatt (1954) in a notable review of the subject reported that investigations in Nigeria indicated that the age-specific mortality figures expressed per 1,000 Africans exposed to risk were as high as 12.5 in infants, 7 in small children, and about 0.5 in older children, adolescents, and adults. On this basis, among the 150 million population some 175,000 children below the age of 15 die each year directly of malaria. What this means as regards morbidity can only be imagined.

Considerable progress in malaria control has been made in the Union of South Africa, Swaziland, in Southern Rhodesia, Madagascar, Mauritius, Réunion, and Egypt but elsewhere the disease is under minimal and often only experimental attack.

TABLE 15

Malaria morbidity and mortality in certain African areas

Country	Year	Malaria Cases	Malaria Deaths
Angola	1952	80,696	292
Bechuanaland	1953	1,865	3
Belgian Congo	1953	279,000	1,190
Cape Verde Islands	1954	6,135	241
French Equatorial Africa	1953	141,001	323
French West Africa	1953	759,310	2,573
Gold Coast	1952	137,265	179
Kenya	1953	7,138	245
Madagascar and Comoros	1953	603,967	307
Mauritius	1942	58,983	3,054
Mauritius	1953	61	2
Mozambique	1950	1,102	30
Nigeria	1952–53	157,701	348
Northern Rhodesia	1953	5,068	98
Nyasaland	1954	50,789	67
Ruanda-Urundi	1953	342,266	604
Southern Rhodesia	1953	3,899	82
Tanganyika	1952	121,757	?
Uganda	1953	91,935	219

Data obtained from reports submitted to the WHO Conference on Malaria in Africa, held at Lagos in November–December, 1955. No claims were made that the malaria reports were complete for the countries concerned.

Mauritius has practically eradicated malaria. The disease was introduced, with the vectors *gambiae* and *funestus*, soon after 1850 with disastrous consequences and prevailed almost unchecked until 1948 when an eradication scheme was started. The first objective was elimination of the vector but this was changed to malaria eradication and has been very closely approached, chiefly through residual spraying, with some larviciding. *Funestus* is now a rare mosquito on Mauritius but *gambiae* remains abundant, although not often taken now in houses. Malaria incidence has dropped to a low level.

In Madagascar prior to 1949 malaria was highly endemic in most areas. A modern control scheme was begun in 1949 and has made great progress. Dwellings are sprayed with residual insecticides, chemoprophylaxis is applied to the entire child population of the island, and larviciding is practiced in urban areas. More than 1.28 million houses were sprayed with DDT in 1954 and some 760,000 children included in the chemoprophylaxis program, using nivaquine (chloroquine). Malaria intensity has sharply declined, as shown by the following data (Joncour, 1955).

Degree of Malaria Endemicity	Number of Districts	
	1949	1955
Hypoendemic	—	50
Mesoendemic	27	30
Hyperendemic	42	—
Holoendemic	11	—
Total	80	80

Réunion has been moderately malarious with spleen rates up to about 30 per cent. The only vector is *gambiae*. A DDT residual spraying project was begun in 1948 and the results have been excellent (Hamon and Dufour, 1954).

Middle Asia (Table No. 16)

Afghanistan: malaria is one of the principal diseases of Afghanistan. The chief vectors have been *superpictus* and *culicifacies*. In Kabul, at 6,000 feet altitude, *superpictus* is a vector of *vivax* malaria from July to September. Data are incomplete, but there have probably been well over a million cases a year on the average. Recently the disease has been well controlled in some areas by residual spraying.

Pakistan: many highly malarious areas are found in Pakistan, particularly in East Pakistan. Case incidence has probably been as high as 25 millions in average years. Control measures have been quite successful in some areas, especially in the West. The vectors are chiefly *superpictus* in the far west, *culicifacies* in Sind, *stephensi* to a limited extent in the south of West Pakistan, *philippinensis* and *sundaicus* in the south of East Pakistan and *minimus* in the north of East Pakistan.

India: there has long been a colossal malaria problem in India with probably 300 millions living in endemic areas. The vectors include all that have been named above for Pakistan excepting *superpictus* and with the addition of *fluviatilis*, a

TABLE 16

Malaria estimates: Middle Asia

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Afghanistan	12,000 (a)	2,500	1,400
Bhutan	300	100 (b)	10 (b)
Burma	19,242	7,500	2,800
Ceylon	8,385	3,000	3,000
India	377,000	300,000	98,000
Malaya Federation	5,889	5,000	4,000
Maldive Islands	88	70	40
Nepal	8,432	3,000	300
Pakistan	80,167	40,116	7,140
Portuguese India	643	111	52
Singapore	1,168	1,100	1,100
Thailand	19,925	10,600	7,300
Totals	533,239	373,097	125,142

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear unless otherwise noted.
3. Malaria data from WHO and personal communications, and also, as noted by author's estimate.
4. (a) 1951; and, (b) author's estimate.

foothill breeder along the Eastern and Western ghats and *leucosphyrus* in parts of Assam. The percentage prevalence of malaria plasmodia in India in 1950 was as follows: *P. vivax* 54.0; *P. falciparum* 40.1; and *P. malariae* 5.9 (Singh *et al.*, 1952). A tremendous nation-wide malaria control project is in progress and it has had notable success (Rao, 1955).

Ceylon: only *culicifacies* has carried malaria in Ceylon and this anopheline, which has been responsible for the principal health problem of the island, is highly amenable to control by residual spraying. A nation-wide malaria eradication project has been highly successful and all 3 millions of those who live in endemic areas are under routine protection either by spraying or by organized surveillance following cessation of spraying. The decline in malaria morbidity is shown in the following table from the 1955 annual report of the Superintendent of the Antimalaria Campaigns.

Year	Estimated Population Millions	Malaria Cases	Morbidity Rate per 1,000
1936	5.63	2,947,555	523
1940	5.95	3,413,618	574
1944	6.28	1,672,478	266
1948	7.10	775,276	109
1952	7.94	269,024	34
1953	8.10	91,990	11
1954	8.39	29,650	3.5
1955	8.59	7,317	0.85

Burma: malaria is a major health problem in Burma where there have been some 6 million cases on the average per year. The chief vectors are *sundaicus* along the coast and *minimus* elsewhere. Widespread control operations are being started.

Maldive Islands: malaria is endemic in the Maldive Islands where *tessellatus* is the vector. Spleen rates up to 70 per cent have been found (Iyengar *et al.*, 1953).

Malaya: malaria has long been one of the principal causes of morbidity and mortality in Malaya but the disease has been under strong attack in Singapore and in urban and estate areas of the Peninsula for over 40 years. Today malaria is uncommon on Singapore Island and there has been an accelerating decline in other areas. Probably at present some four-fifths of the 5 millions living in malarious areas are under routine protection. The chief vectors have been *sundaicus* in brackish sunlit pools and drains along the coast, *maculatus* in sunny foothill streams, springs, and seepages, and *umbrosus*, *letifer*, and *barbirostris* on the flat coastal plains in slow, stagnant water, often shaded.

Thailand: at least 10.6 millions of Thailand's nearly 20 million population live in areas where malaria is endemic. But over 7 millions are now under routine protection by residual spraying which has proved quite successful, particularly in areas where *minimus* is the vector.

Far Eastern Asia (Table No. 17)

Indochina: Cambodia, Laos, and Vietnam have always been highly malarious and remain so. Political upheavals have delayed the application of modern control measures. Some 1.2 millions of the approximately 7 millions living in endemic areas are under some protection from malaria at the present time. Malaria is especially abundant in foothill regions where *minimus* is the vector. Other species that have been found naturally infected are *sundaicus*, *sinensis*, and *tessellatus*. Malaria prevalence is highest from July to September and December to March, at the beginning and end of the monsoon rains. The great deltas of the Mekong and Red Rivers are relatively free of the disease.

China—mainland: no reliable data are available as to recent malaria incidence and distribution in Communist China. In the past, malaria was not found north or west of a line running southwards from Wanchuan (Kalgan), Central Shensi and southern Shensi, then running westwards through central Kansu. The disease was highly endemic from the lower Yangtze valley extending southeast to and including Hainan; in southwest Yunnan, especially adjacent to Burma; in the upper Yangtze valley; and in the Amur River basin. Elsewhere in Manchuria endemicity was slight as also in Hunan Province. The foothills of South China have always been highly malarious due chiefly to *minimus*. The less intense malaria of the plains of South China has been maintained by the ricefield breeder *sinensis*. In North China *pattoni* has been incriminated and on the high plateau of Northwest China, *sacharovi* has been involved (Covell, 1949).

Visitors to Communist China in 1955 report having seen a malaria control center in Szemao, southwest Yunnan Province, where successful operations included environmental sanitation, drainage, insecticidal spraying, and in selected spots, the use of quinine and chlorguanide (Singh, 1956, personal communication).

TABLE 17

Malaria estimates: Far Eastern Asia

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Brunei	55	40	32
Cambodia	4,100	1,500	180
China (mainland)	582,603	200,000 (b)	10,000 (b)
Hong Kong	2,277	2,277	2,277
Indonesia	81,100	30,000	5,277
Japan	88,000	517	388
Korea	30,000 (a)	5,000	500
Laos	1,360	1,000	10
Macau	200	75	50
Mongolian Peoples' Republic	920	0 (b)	—
Netherlands New Guinea	700	700	80
North Borneo	364	290	24
Philippines	21,440	6,300	6,000
Ryukyu Islands	778	40 (b)	40 (b)
Sarawak	602	550	50
Taiwan (Formosa)	8,617	5,640	5,640
Timor	466	200	50
Vietnam	26,000	4,500	1,000
Totals	849,582	258,629	31,598

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from WHO and personal communications, and also, as noted, by author's estimate.
4. (a) 1953; and, (b) author's estimate.

Macau: prior to World War II malaria was an important problem in Macau. The chief vector has been *minimus* with assistance from *sinensis* and the main breeding places have been located just across the border in China. There has been a sharp decline since 1950. Hospitals and dispensaries have reported malaria as follows (Dy, 1956, personal communication).

1950	1,092 cases	45 deaths
1951	570	6
1952	510	8
1953	164	4
1954	89	4

The reduction in incidence indicated by hospital reports but not yet by surveys, has been due in part to restriction of traffic with China and in part to control by residual spraying with DDT and BHC.

China—Taiwan: Formosa has long been highly malarious with *minimus* and *sinensis* the chief vectors. An energetic control project in recent years has

greatly reduced the incidence. Very likely some 5.64 of the 8.617 millions in Formosa live in endemic areas and practically all of those exposed are now under protection by residual spraying.

Japan: malaria has never been common in Japan although there are some *vivax* endemic areas in the southern islands where *sinensis* is the vector. Some localized epidemics followed the return of civilians and soldiers at the end of World War II. The disease is only mildly endemic in Okinawa.

Korea: malaria is highly endemic in southern Korea but much less so in the north. The vectors are *minimus* and *sinensis*.

Indonesia: the archipelago of the Republic of Indonesia is highly malarious, the chief vectors being *sundaicus* along the coast, and *aconitus* in rice fields, with assistance from *maculatus* in some areas. In Sumatra, *sinensis* plays a part, and in the Celebes *farauti* is a vector, while in Borneo *leucosphyrus* probably is chiefly responsible. A nation-wide control project is in progress. Some 5.28 millions of the 30 millions exposed are now under protection.

The Philippines: malaria in the Philippines is a disease of the foothills below 2,000 feet altitude and transmitted by *minimus*, *flavirostris* and *mangyanus*, found chiefly in small streams. The coastal areas are entirely free of the disease unless the foothills are close by. It has been estimated that 2 to 4 million cases occurred annually, with 10 to 20,000 deaths from malaria. Financial losses due to this disease have been put at $100 million or more per year. But a nation-wide eradication project has already reduced the incidence markedly in some areas and there is good reason to believe that the disease will be completely controlled in the next few years (Ejercito *et al.*, 1954).

Hong Kong: malaria is endemic in Hong Kong where *minimus* and *jeporiensis candidiensis* are the chief vectors with assistance from *maculatus*, and *sinensis*. But today the disease is under firm control.

British Borneo: malaria prevalence in Sarawak and Brunei is surprisingly low in many areas. It is highly endemic in the hilly and mountainous sections. A WHO survey by Zulueta showed that the seminomadic Dayak, Kayan, and Kenyah, who make temporary shelters each year while planting their rice, are more malarious than the Chinese and Malays. Inland rural Malays are more often infected than those who live along the coast or great rivers. The vector in Sarawak and Brunei is *leucosphyrus leucosphyrus* and in British North Borneo, *leucosphyrus balabacensis*. Sporozoite infections in *A. l. balabacensis* have been found as high as 10 per cent in the Tambunan district by Chang, a WHO entomologist. Among some 7,568 *A. l. leucosphyrus* dissected by the WHO unit in Sarawak, there was a sporozoite index of 0.40 per cent. *Barbirostris* is occasionally a secondary vector in Sarawak (Dy, 1954, 1955).

Australia and Oceania (Table No. 18)

Australia: malaria today is only mildly if at all endemic on the Australian mainland. There are possibly some residual foci along the northeastern coast of the Northern Territory and in the northern projection of Queensland. Foci of *falciparum* malaria, transmitted by *farauti*, are known in the Torres Straits Islands,

TABLE 18

Malaria estimates: Oceania

Locality	Population	Population in Malarious Areas	Population Routinely Protected
Australia	8,987	100	80
Australian New Guinea	1,207	1,100	7
Fiji Islands	328	0	—
French Oceania (a)	45 (b)	0	—
Gilbert and Ellice Islands	40	0	—
Guam	35	0	—
Hawaii	522	0	—
New Caledonia	21 (b)	0	—
New Hebrides	50	48	6
New Zealand	2,093	0	—
New Zealand Oceania (c)	23	0	—
Papua	495	375	3
Samoa (U.S.)	114	0	—
Solomons	103	96	1
Tonga	52	0	—
U.S. Pacific Islands (d)	61	0	—
Western Samoa	92	0	—
Totals	14,268	1,719	97

Notes: 1. Figures in thousands.
2. Population data from *U.N. Demographic Yearbook*, 1955. These are estimated 1954 midyear, unless otherwise noted.
3. Malaria data from WHO and personal communications.
4. (a) Austral, Gambier, Marquesas, Rapa, Society and Tuamoto Islands; (b) 1937; (c) Cook, Niue, and Tokelau Islands; and, (d) Carolinas, Marianas, and Marshall Islands.

especially in the Western group. In the past the disease occurred in Western Australia as far south as Fitzroy Crossing at latitude 18° S., often in epidemic form; in the Northern Territory it was endemic as far as Wave Hill at latitude 17° 30′ S.; and in Queensland it reached 19° S. on the coast (Black, 1954; Ford, 1954, personal communications).

Oceania: Anopheles farauti is an important vector in New Guinea, the Solomons, Hebrides, and from New Britain to the Eastern Celebes. It frequently transmits when feeding out-of-doors. Also an effective transmitter is *punctulatus* in New Guinea, and from the Solomons to Halmahera. In Netherlands New Guinea, *karwari* is also a vector. Malaria is highly endemic in the New Hebrides (except Futuna Island), the Trobriands, New Britain, New Ireland, the Admiralty Islands, New Guinea, and the Solomons. All three plasmodia, *vivax*, *falciparum*, and *malariae* are present. Not much control is in progress. Experiments with DDT residual spraying in Netherlands New Guinea have given promising results.

CONCLUSIONS

A world survey of malaria and its control reveals that there has been a tremendous acceleration in the attack on this disease during the past few years and that for many countries eradication is technically and economically practicable within the next ten years. But the data, imperfect though they are, refute the notion that the sun has now set on malaria and malariologists. One might more truly say that the morning's work has been well started but that it is not yet high noon for malariology. There still remains an exceedingly large task involving both research and control on a higher rather than a lower scale.

It seems logical to close this review with a list of some of the principal needs in the world-wide campaign against malaria.

1. Better measurement of the effect of malaria eradication on the health, social, and economic status of a community.

2. More effective measures for eradicating malaria among primitive and relatively inaccessible peoples in such areas as exist, for example, in parts of Amazonia, Borneo, and New Guinea.

3. Basic investigations of African *A. gambiae* to clarify its position as a species or as a complex and to determine the behavior characters of the insect as they relate to malaria and its eradication.

4. Clearer understanding of the prime causes and practical implications of anopheline resistance to insecticides.

5. More effective and frequent use of antimalaria therapy to eradicate the parasite from communities.

6. Practical measures for dealing with the terminal phases of nation-wide eradication projects as regards measures both of attack and of assessment.

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THE TIME REQUIRED TO REACH ERADICATION IN RELATION TO MALARIA CONSTITUTION

ARNOLDO GABALDON

División de Malariología, Dirección de Salud Pública, Ministerio de Sanidad y Asistencia Social, Maracay, Aragua, Venezuela

Malaria control and finally eradication of the disease by residual insecticides is generally obtained by killing the anopheline vector before it reaches the infective stage (Gabaldon, 1948, 1953). Depending on the bionomics of the vector species, malaria may respond in different ways to the effects of the insecticide. When the vector is an indoor-biter and an indoor-rester, the disease is responsive to the use of the chemical, but if it bites and rests outdoors, the disease is refractory to house spraying. When some of the mosquitoes are reached by the insecticide but others escape its effect by biting or resting out of doors, we may have partially refractory malaria (Gabaldon, 1953). But even in areas where the disease is responsive to the insecticide, different degrees of transmission may be present, and the more frequent this is, the more difficult it may be to interrupt. Because of these facts it was considered appropriate to review from this standpoint the data of the first large area of malaria eradication in Venezuela.

This area, called Area I, has 180,000 square kilometers, and is the largest area where malaria has been eradicated in the tropical zone (Gabaldon and Berti, 1954, 1955). It is made up of 284 municipalities, of which only 25 did not have malaria. It occupies the north-central portion of Venezuela and forms part of the Costa-Cordillera and the Llanos regions of this country. Here malaria yielded to DDT. This area displayed all the epidemiologic types of malaria present in Venezuela and therefore may give information on the problems mentioned above. No primary indigenous cases of malaria have been found here in the last four years.

The nation-wide campaign against malaria in Venezuela, begun in 1945, was based on DDT indoor residual spraying, and had as its aim "the eradication of malaria from Venezuela" by "protecting all the houses of the malaria zone, even those of sparsely populated or mildly malarious districts" (Gabaldon, 1949). It was the first nation-wide campaign organized with such an objective (Russell, 1952). The initial plan contemplated having the whole of Venezuela malaria-free by 1955 by spraying 100 per cent of the houses of the infected zone. This plan, however, was not feasible due to circumstances explained elsewhere (Gabaldon and Berti, 1955). Nevertheless, it is believed at the present time that nothing except lack of funds should prevent the attainment of the original goal.

CONSTITUTION OF MALARIA

There are two facts which it is important to know about the malaria in any region: (a) the quantity of the disease present, and (b) its variability under

natural circumstances. The quantity of malaria represents its endemicity, and the variability of the disease is a sign of its epidemicity. The conditions governing the endemicity and the epidemicity of the human plasmodial infection in a given area make up the *constitution of malaria* in such area.

The spleen index has been used as a measure of the quantity of malaria present at the period when the examination is made, but if spleen indexes are made for several consecutive years in a locality at the same season, they also measure the variability of this quantity. In this case, the spleen indexes serve to determine the constitution of malaria, and to measure the degree of endemicity and epidemicity of the disease.

Some children in non-malarious localities have slightly enlarged spleens, and some authors therefore consider an index of 5 per cent as "normal". The lowest spleen index of a malarious community over five to seven years is presumably the measure of the lowest degree of transmission which may take place in that community. If this is divided by 5, the figure corresponding to the normal spleen index, the quotient represents a measure of the least amount of malaria possible under local conditions. This quotient has been called the *ratio of endemicity*, and is a measure of the minimum prevalence of the disease. As malaria tends to show a quinquennial periodicity, the largest spleen index in such a cycle, divided by the smallest, will indicate the degree of fluctuation suffered by the disease in the cycle. This quotient has been called *ratio of epidemicity*, and is a measure of the maximum variability of malaria. These two ratios, and the concept of constitution of malaria (originally called "condition"), were introduced 10 years ago by the writer and published later with additional comments (Gabaldon, 1949).

Where transmission is frequent throughout the year, the quantity of malaria is uniformly high, and there is minimum variability. These are areas of high endemicity and low epidemicity. In districts where transmission is, on the contrary, uniformly infrequent year after year, both endemicity and epidemicity are low. Where transmission decreases in some years and increases in others, endemicity depends on the lowest level reached, and epidemicity on the degree of the increase. Therefore, endemicity and epidemicity as here defined are terms which characterize the constitution of malaria, and the values as given by their respective ratios, are inversely related, as shown in Table 1. This concept of malaria epidemicity as dependent on the variability of transmission is very similar to the "malaria instability" of Macdonald (1952)—malaria of low epidemicity being relatively stable and that of high epidemicity unstable, with many intermediate situations.

In Table 1 are presented the ratios of endemicity and epidemicity and the spleen indexes of some localities in the area of malaria eradication in Venezuela called Area I (Gabaldon and Berti, 1954, 1955). These localities are divided into three groups according to their malaria constitution. In Group I, there is high endemicity and low epidemicity as shown by the permanently large spleen indexes. In Group II, endemicity is low and epidemicity is high, with a marked variability in malaria prevalence. Group III is characterized by low endemicity

TABLE 1

Ratios of endemicity and epidemicity, spleen indexes, and vectors of some Municipalities of the first large area of malaria eradication in Venezuela

Municipality	First Sprayed	Ratios of		Spleen Indexes Before 1946		*A. albimanus*	*A. darlingi*	*A. albitarsis*	*A. aquasalis*	*A. Pseudo-puncti-pennis*
		Endemicity	Epidemicity	Smallest	Largest					
Group I: High endemicity and low epidemicity										
Carmen de Cura, Aragua	4/1946	20	1	100.0	100.0	−	+	−	−	+
Taguay, Aragua	4/1946	20	1	100.0	100.0	−	+	+	−	+
Camatagua, Aragua	4/1946	19	1	95.8	100.0	−	+	+	−	+
Onoto, Portuguesa	1/1947	18	1	88.8	90.9	−	+	+	−	+
Ospino, Portuguesa	2/1947	16	1	79.5	92.6	−	+	+	−	+
Urama, Carabobo	12/1945	15	1	76.6	92.8	+	+	+	−	+
El Pao, Cojedes	1/1947	15	1	76.0	94.7	−	+	+	−	+
Morón, Carabo	12/1945	14	1	72.7	98.6	+	+	+	+	+
Libertad, Cojedes	2/1947	14	1	72.2	90.3	−	+	+	−	+
Guardatinajas, Guárico	3/1947	14	1	71.7	100.0	−	+	+	−	−
Ortiz, Guárico	11/1946	13	1	65.8	87.5	+	+	+	−	+
Parapara, Guárico	10/1946	12	2	58.3	96.6	−	+	+	−	+
Cojedes, Cojedes	12/1946	10	2	50.0	91.0	−	+	+	−	+
Median		15	1	76.0	94.7					
Group II: Low endemicity and high epidemicity										
Cagua, Aragua	3/1946	1	12	2.0	60.8	+	−	+	−	+
Tinaquillo, Cojedes	5/1947	1	11	2.8	56.7	−	+	+	−	+
San Diego, Carabobo	8/1946	1	11	5.6	63.6	+	+	+	−	+
Macapo, Cojedes	10/1947	2	9	7.9	74.0	−	−	+	−	+
Naguanagua, Carabobo	11/1947	1	9	2.6	46.2	−	+	+	−	+
Borburata, Carabobo	11/1946	1	8	6.8	53.2	+	+	−	+	+
San Joaquín, Carabobo	7/1946	1	7	0.8	34.6	+	+	+	−	+
Güigüe, Carabobo	6/1946	1	7	3.5	37.2	+	+	+	−	+
La Victoria, Aragua	3/1946	1	6	3.4	31.1	+	−	+	−	+
San Sebastián, Aragua	3/1948	2	6	8.5	40.9	−	+	−	−	+
Patanemo, Carabobo	4/1947	3	6	16.0	88.9	+	+	−	−	+
El Baúl, Cojedes	1/1947	2	5	7.7	40.2	−	+	+	−	−
La Pascua, Guárico	11/1947	2	5	7.8	41.3	−	+	+	−	+
Altagracia, Guárico	6/1947	2	5	9.9	46.8	−	+	+	−	+
Median		1	7	6.2	46.5					

Group III: Low endemicity and low epidemicity

Tucupido, Guárico	7/1947	3	3	14.4	39.4	+	+	+	−	+
Sta. Teresa, Miranda	6/1948	3	1	13.1	13.3	+	−	−	−	+
Naiguatá, Dto. Federal	11/1947	2	2	9.1	17.8	+	−	−	+	+
Chivacoa, Yaracuy	1/1947	2	2	8.8	17.6	−	+	−	−	+
Santa Lucía, Miranda	4/1947	2	1	11.8	15.4	−	+	−	−	+
San Fco. Asis, Aragua	11/1946	1	3	7.1	20.0	+	−	−	−	+
Sarare, Lara	5/1948	1	3	5.1	14.1	+	+	+	−	+
San Javier, Yaracuy	10/1946	1	3	6.1	19.0	−	+	+	−	+
Cazorla, Guárico	6/1948	1	2	1.9	10.3	−	−	+	−	−
Guama, Yaracuy	12/1946	1	2	1.6	12.4	−	+	−	−	+
San Felipe, Yaracuy	12/1946	1	2	6.3	14.8	−	+	+	−	+
Yaritagua, Yaracuy	1/1947	1	2	3.6	12.3	−	+	+	−	+
Median		1	2	6.7	15.1					

and epidemicity. These groups clearly show how unsafe it is to judge malaria prevalence by one spleen index alone. The ratios of endemicity and epidemicity distinctly indicate that the constitution of malaria was different in these three groups, and that there may also be intermediate stages between them. Indeed, the localities included in each group were especially selected as typical examples of the extreme cases they represent. Many localities of Area I were discarded for the purposes of this study because they were intermediate in their malaria constitution.

The facility of interruption of transmission by indoor residual spraying should depend on the constitution of malaria. In a locality with low ratios of endemicity and epidemicity and relatively few infected vectors, one DDT application may be sufficient to stop transmission almost entirely, while in a highly endemic locality where infected vectors are much more numerous, some may survive and transmission continue in spite of the spraying, although at a lower level. If this assumption is correct, a locality with low endemicity may reach malaria eradication in a shorter time than a locality with high endemicity. It would obviously be advantageous to attack malaria of the epidemic type during the interepidemic period when the number of infected vectors is reduced.

Furthermore, in a locality with low ratios of endemicity and epidemicity the average infections should be of longer duration than in localities with high ratios of endemicity. In the latter, all the infections found may not be older, theoretically, than twelve months if every person receives one or more infective bites per year. In localities with low endemicity the infections found are made up of both primary attacks and relapses, some of which are due to infective bites received during the previous year or years. In this case, if transmission is stopped, these older infections will tend to die out more quickly than in localities where most of the infections are recent. This is another reason why malaria in areas of low endemicity and low epidemicity may be eradicated in shorter time than in areas of different malaria constitution.

MALARIA ERADICATION AND MALARIA CONSTITUTION

Spleen and parasite indexes are the measures of malaria prevalence ordinarily used in control programs. When the nation-wide campaign against malaria began in Venezuela, it was evident that if the goal was eradication of the disease, a measure of the morbidity with microscopic confirmation of cases would be required. But morbidity rates as used in the regular epidemiologic services were not obtainable under Venezuelan conditions where medical care is unevenly distributed, particularly in the zones infected with malaria. For this reason a morbidity index was introduced based on the data collected by the health visitors who work mostly in rural districts. These men take a blood smear from every person found with fever at the time of the visit, or who reports having had fever during the previous week. In their work they usually follow the itinerary of the spraying squads, so that most of the treated houses receive one or more visits per year. For some years after the beginning of the DDT program, this morbidity index, called "positive slides per 100,000 house visits",

was found to be adequate (Gabaldon, 1949). But the activities of the health visitors, both before and after this program began, were confined mainly to epidemic areas in order to treat fever cases, while areas of high or low endemicity where the number of acute cases was smaller, received fewer visits. Due to this fact, the available figures, although useful for making comparisons between whole areas before and after the DDT program, do not suffice when such regions are divided into zones according to the degree of endemicity and epidemicity.

Since, in an eradication program, no possible source of discovering malaria cases may be omitted, laboratory facilities were offered to practicing physicians so as to obtain microscopic confirmation of all cases reported by them as malaria. In Area I, since 1951, all reported cases have received microscopic diagnosis (Gabaldon and Berti, 1954, 1955). The last indigenous cases found by this means occurred in the same year (1951) as those found by health visitors, so that the figures given by these workers may in fact be used to indicate the end of transmission.

Table 2 indicates the progress of eradication in the three groups of municipalities presented in Table 1. Group I, comprising the municipalities with high endemicity and low epidemicity (Table 1), had no reported cases in the fifth year after the first spraying (Table 2). There was an increase in the number of positive slides per 100,000 house visits in this group from 873 in the first year to 1,481 in the second, but this was apparently due to the increase in the number of visits that the group received in the second year, which were concentrated on the most infected districts. From here on, the morbidity index fell sharply. In Group II, municipalities with low endemicity and high epidemicity, the decrease was slower but cases also reached 0 in the fifth year. It is hard to explain the difference in the rate of decrease of these two groups. But since, in a nationwide campaign, it is not possible to organize an epidemiologic service with optimum efficiency everywhere, the difference observed was probably due to errors of sampling in the visited areas in successive years. In any case, both groups reached zero in the fifth year after the first spraying. These two groups had had, at least for some years, equally intense degrees of transmission. In contrast Group III, comprising the municipalities with low endemicity and low epidemicity, had no reported cases by the third year after the first spraying two years earlier than the first two groups. This may be explained by the fact that probably in Group III transmission was totally interrupted by the first sprayings, while in Groups I and II some new infections were produced, at least toward the end of the six-months spraying cycle, as was shown to be the case in other parts of Venezuela (Gabaldon, 1951, Table 7). Furthermore, as the proportion of new infections every year must have been relatively higher in Groups I and II than in Group III, the duration of these new infections may also be responsible for the longer time taken to reach eradication in the first two groups.

With regard to the different species of plasmodia, some facts are of interest. The three groups of municipalities differed markedly in this respect before DDT spraying (Table 3). If the data for the three groups were quantitatively

TABLE 2

Time required for malaria eradication in three groups of municipalities of Venezuela according to their malaria constitution

Group of Municipalities	Years After First Spraying*				
	First	Second	Third	Fourth	Fifth
Number of house visits made by rural visitors					
Group I	4,469	7,157	8,914	9,097	8,662
Group II	18,079	17,768	12,645	14,015	14,979
Group III	19,746	18,298	18,873	14,584	15,366
Positive slides per 100,000 house visits					
Group I	873	1,481	415	55	0
Group II	183	22	32	7	0
Group III	86	27	0	0	0
Percentage of single infections with *P. falciparum*					
Group I	49	56	51	0	0
Group II	47	25	75	0	0
Group III	39	0	0	0	0
Percentage of single infections with *P. vivax*					
Group I	51	37	49	100	0
Group II	47	75	25	100	0
Group III	50	100	0	0	0
Percentage of single infections with *P. malariae*					
Group I	0	7	0	0	0
Group II	7	0	0	0	0
Group III	11	0	0	0	0
Percentage of positives slides with mixed infections					
Group I	5	0	3	0	0
Group II	0	0	0	0	0
Group III	0	0	0	0	0

* Calendar years following the year of the first spraying.

not suitable for the comparisons mentioned above, they are dependable as to the relative distribution of the species of malaria parasites. The prevalence of *P. vivax* was lower in Group I and higher in Group III, the reverse of that of *P. falciparum*. There is no marked difference in the prevalence of *P. malariae* in the three groups, although, considered by itself, it was twice as common in Group III as in Group I. The percentage of mixed infections decreased from Group I to Group III. In all these comparisons Group II occupied an inter-

TABLE 3

Parasite formula of single infections and percentage of mixed infections of the three groups of municipali'ies (pooled data 1941–1945)

Species	Group I	Group II	Group III
P. vivax	35.4	41.6	55.9
P. falciparum	59.8	51.8	35.9
P. malariae	4.9	6.6	8.1
Mixed infections	15.3	13.7	8.7

mediate position, its difference from Group III being more marked than its difference from Group I.

All these findings are in agreement with the known facts of the prevalence of the malaria plasmodia in relation to the degree of endemicity of the disease. After spraying, *P. falciparum* disappeared from the three groups a year ahead of *P. vivax*. The extinction of *P. falciparum*, however, was not gradual as used to be the case when malaria control was carried out by drainage, but was rather abrupt. *P. malariae* disappeared even earlier in spite of the known fact that it produces the longest infections. Mixed infections however were the first to be extinguished. Group I, with the highest percentage of mixed infections, was the last to show them. It may be said that they become extinct during the first year after the first spraying, because the 3 per cent of mixed infections in Group I during the third year was due to only one mixed infection among the 36 positive slides collected that year in the group. This should be useful in evaluating the efficiency of spraying as the persistence of mixed infections will be a clear sign that transmission has not been sufficiently interrupted by the insecticide.

DISCUSSION

The findings presented above show definitely that eradication is reached at different times according to the degree of endemicity and epidemicity, that is, the constitution of malaria, in a given region. To have an adequate understanding of the malaria constitution is therefore useful in forecasting the probable time required for an eradication program. This applies naturally to areas where the disease is responsive to the insecticide. But as this character depends on the vector or vectors present, an appropriate entomological study should accompany the basic plans for a program of this type.

Malaria was responsive to indoor residual insecticide spraying in Area I and sectors of other areas from which it has been eradicated in Venezuela. The major vectors in Area I were *A. darlingi* and *A. albimanus*. *A. albitarsis*, *A. aquasalis*, *A. núñez-tovari* and *A. pseudopunctipennis* were also present, but with the exception of *A. albitarsis* in a limited district, they played a minor role if any. *A. aquasalis* in Area I lays an egg similar to the one described from Panama, but different from the egg of the malaria-carrying form present in Eastern Venezuela, West Indies, and Brazil. This species therefore probably did not play any role in transmission in Area I. *A. núñez-tovari* was an occasional finding

and did not show densities high enough to be of importance. *A. pseudopunctipennis* probably transmitted some malaria where it coexisted with *A. albimanus* or *A. darlingi*, but where it was alone only sporadic infections were found, which disappeared without DDT as soon as the arrival of infected people from really malarious areas was stopped. In municipalities of high endemicity and low epidemicity (Group I) *A. darlingi* was the main vector (Table 1). In municipalities with low endemicity and high epidemicity (Group II) *A. albimanus* or *A. darlingi*, or both, were present and showed irregular fluctuations in density which were responsible for the epidemic outbreaks (Gabaldon, 1948). In municipalities with low endemicity and epidemicity (Group III) *A. albimanus* and *A. darlingi* when present were in very low densities.

It is important to observe that *A. albimanus* alone was never responsible for situations of high endemicity and low epidemicity, such as those produced by *A. darlingi* in Group I. Malaria of this epidemiologic type does not occur in Mexico, Central America and the Antilles where *A. albimanus* is the major vector; at least that is the conclusion reached from a careful study of the published literature. Even in Haiti, reputed to be one of the most malarious countries in the Western Hemisphere, the published figures do not substantiate the statement made by Paul and Bellerive (1947) that the situation there is similar to that found in areas of equatorial Africa. Holoendemic malaria of the African type does not exist in the Americas, so far as the known data indicate. The Haitian conditions mentioned above may very well be of the type of our Group II municipalities, characterized by median endemicity and epidemicity, since we know that from one spleen index alone no judgment can be made as to the malaria constitution. The fact that *A. albimanus* does not produce such conditions in Venezuela supports this conclusion. This is a very fortunate situation, as it indicates that malaria eradication in those countries should not be difficult. Indeed, Venezuelan data show that in municipalities where *A. albimanus* alone is the vector, eradication may be reached by the third year after the first spraying, even in Group II municipalities (Gabaldon and Berti, 1955).

It was the Venezuelan experience that *darlingi*-transmitted malaria of high endemicity or high epidemicity took the longest to eradicate. It is to be expected therefore that the continental program of malaria eradication may find more difficulties in the *darlingi* areas of South America than in other parts of the Western Hemisphere. This may prove to be the case in Colombia, where the severity of the epidemiological types approaches that of Venezuela and the Guianas. Further south, there is a decrease in the incidence of *P. falciparum* (Gabaldon, 1948), a sign of lower intensity in transmission.

However, there is another problem which may be of paramount importance as a formidable obstacle to eradication. This is the existence of partially refractory malaria. *A. aquasalis* (where it is a vector) and *A. núñez-tovari* often bite and rest out of doors and are not all affected by indoor residual spraying, so that transmission can continue in spite of an efficiently operated program (Gabaldon and Berti, 1954, 1955). We do not know the distribution of the vector strain of *A. aquasalis* in the Southern Continent, and suspect that it

may be a local form of the species. But *A. núñez-tovari* is extensively distributed in South America (Gabaldon and Cova-García, 1952) and is a confirmed vector in Colombia (Rey and Rengifo, 1950). If it produces partially refractory malaria throughout its area as it does in Venezuela, great difficulties lie in the way of eradication. Furthermore, in certain areas of Brazil, *A. darlingi* is also in part an outdoor-biter and outdoor-rester and interruption of transmission has not been so easily achieved by DDT spraying (Bustamente, 1951). Fortunately, outside the Amazon countries where these two last-named species are present, the problem of malaria eradication seems to be more feasible.

Since Venezuela is at the crossroads of the neotropical anophelines, the vectors found here belong to the Mexican and Brazilian subregions, and the experience obtained in this country should be useful to the other republics now engaged in the continental program of malaria eradication. With the exception of some effective vectors of the subgenus *Kerteszia*, which in this country do not reach the densities required for transmission, all the other neotropical vectors of importance are found here. The main malariological factor inhibiting eradication in Venezuela has been the presence of partially refractory malaria due to *A. aquasalis* and *A. núñez-tovari*. In the sectors where insecticides alone are unable entirely to curb transmission, drugs have been used with success.

SUMMARY

In Venezuela malaria has in some areas been responsive, and in others partially refractory, to DDT indoor residual spraying. Eradication has been achieved in the former areas, as for example in the great north-central region (Area I), where indigenous cases have not been found since 1951. The time required for eradication after the beginning of a spraying program depends on the constitution of malaria, that is, the degree of endemicity and epidemicity of the disease peculiar to a given region. In zones of high endemicity and those of high epidemicity, eradication was reached by the fifth year. In zones of low endemicity and low epidemicity, no more cases were observed after three years of spraying.

The time needed for eradication depends mainly on the bionomics of the vector. *A. darlingi* malaria took five years to disappear whereas *A. albimanus* malaria required only three. Where both species occurred together in low densities, three years proved sufficient to eliminate malaria.

As Venezuela is at the crossroads of the neotropical anophelines, and most of the important vectors are present in the country, the experience presented here may be useful for the projected continental program of malaria eradication.

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A PILOT PROJECT OF RESIDUAL-INSECTICIDE SPRAYING TO CONTROL MALARIA TRANSMITTED BY THE *ANOPHELES PUNCTULATUS* GROUP IN NETHERLANDS NEW GUINEA

D. METSELAAR

Director of the Malaria Division of the Public Health Service of Netherlands New Guinea

New Guinea is situated in the Australasian region between 130°–152° E. longitude, and 0°–12° S. latitude, and belongs therefore to the real tropics. It is divided by the 141st meridian into an Eastern or Australian, and a Western or Dutch part. There is a central mountain range with altitudes up to 5,000 meters, with mountains and marshy plains alternating between this and the coast. The lower altitudes are as a rule highly malarious and transmission of malaria is perennial, except for certain areas in the south where, between May and October, a dry season temporarily prevents the breeding of mosquitoes.

The main vectors are *Anopheles punctulatus*, *A. farauti* and *A. koliensis*, while locally *A. bancrofti*, *A. karwari* and *A. subpictus* may play a part.[1] *A. clowi* has so far not been encountered in sufficient numbers to be of epidemiological importance. Of these, the 3 members of the *punctulatus* group are very indiscriminate in the choice of breeding places. When the water surface is sunlit, they breed almost everywhere and have been collected from fresh and brackish water, hoofprints and vast marshes, standing water and along the banks of brooks and rivers. *A. punctulatus* prefers fresh temporary rainpools, often without vegetation, while *A. farauti* and *A. koliensis* larvae are collected mainly from permanent water collections with vegetation, or at times from brackish water of high salt content.

Anti-larval methods of malaria control can only be applied on a very limited scale because of the variety and quantity of breeding places. These species at certain times may occur abundantly, and a hundred anopheline bites per unprotected person per night are not uncommon; but during relatively dry periods these numbers may go down to ten or even less. Members of the *punctulatus* group are highly anthropophilic, but do not refuse animal blood. Though adult mosquitoes have been collected indoors by day and night, their principal resting places are out of doors. At Salamaua, Roberts and O'Sullivan (1948) have noticed a preference for indoor biting, but it is a current opinion that these mosquitoes prefer biting out of doors. Thus malariologists have been sceptical about the residual action of DDT against *punctulatus* anophelines, and this possibly explains why, until recently, only small scale trials to test the value of indoor spraying with insecticides have been made. Yust (1947), in the New Hebrides, reported that 5 per cent DDT residual spray applied to native huts controlled *A. farauti* for three months; however the breeding places were also treated. An unpublished

[1] The nomenclature proposed at the Kampala Conference in Equatorial Africa is used in this publication, though there is reasonable doubt whether it can be applied to New Guinea conditions (WHO, 1951).

U. S. Army report, quoted by Roberts and O'Sullivan (1948), speaks of encouraging results in Salamaua, where the three main species of the *A. punctulatus* group occur. Swellengrebel and Stack (1948 unpublished) painted a few houses near Sentani Lake with DDT in kerosene, but obtained inconclusive results. Bang *et al.* (1947) sprayed a village in the southern part of Australian New Guinea, 150 miles west of Port Moresby, where *A. punctulatus moluccensis* and *A. subpictus* were the prevalent anophelines, using 4 per cent DDT in kerosene at a rate of 1.0 gram per square meter. After 4 months the parasite rate had slightly decreased, while the spleen rate remained the same. In an untreated control village both had increased. On the strength of these observations, it was emphasized at the First Asian Malaria Conference in Bangkok in 1953 that a new experiment, which would comprise a larger area and where observations could be continued over a longer period, seemed necessary.

The results, observed one year after the first spraying in such an experiment, are the subject of this publication. Part of this pilot project consisted of trials with window-trapped houses to obtain more information on the biting and resting habits of the local anophelines. Later, 2 of the 3 houses were sprayed with DDT (2 gm./m.^{2}) and dieldrin (0.5 gm./m^2) respectively. The results of this trial, including those of an observation period of 6 weeks after the insecticide application, have already been published.

The vector anophelines have a tendency to stay indoors for a longer period than was expected, though *A. farauti* preferred to bite man out of doors, while *A. koliensis* could be collected more easily on human bait inside a house than out of doors. A satisfactory kill was obtained by the insecticides (Van Thiel and Metselaar, 1954; 1955). Prolonged observation made it clear that from 25 to 30 weeks after application, 60 per cent of the anophelines were killed within 24 hours by the insecticides in the sprayed houses as compared with only 14 per cent in the window traps of the control house. Then the number of anophelines decreased considerably due to an abnormally low rainfall, and the observations were interrupted. Later, all the houses for miles around were sprayed, with so much contamination with DDT everywhere that even the mosquitoes in the window traps of the unsprayed control house died and further observations became of no value.

THE EXPERIMENTAL AND CONTROL AREAS. GEOGRAPHY AND COMMUNICATIONS

The experimental area is situated southwest of Hollandia in the basin of Sentani Lake, which on the northern side is separated from the Pacific Ocean by the Cycloop Mountains (2,360 meters) and on the three other sides is surrounded by hills not higher than 550 meters. The elevation of the lake itself is about 70 meters above sea level. Along the northern coast, between the lake and the Cycloop Mountains, are some plains, the lower parts of which form extensive sago marshes, and along the other sides many smaller marshes form an equal number of potential breeding places. For practical reasons in relation to this experiment, the Sentani basin will be divided into the "lake area" or experimental area in the proper sense, and the "surrounding area."

The lake itself is about 30 km. long and 15 km. wide. It derives its water from the many brooks and rivers coming down from the hills and the mountains. In the southeast corner is a narrow break in the ring of mountains and hills through which the surplus water runs off. However, in periods of heavy rainfall the water surface of the lake may rise about one meter, flooding the surrounding marshes and favoring the breeding of numerous anophelines.

A road from Hollandia to the Tanahmerah Bay runs along the northern side of the lake, while a ferry crosses it from east to west, and connects with a jeep road, enabling us to reach the control area, situated about 25 km. westerly in the centre of the Nimboran plain, which forms the western part of the Grimé Plain, a vast valley covered with many marshes. A motor launch of the Public Health Service is at our disposal when we need it for experimental work on the lake.

Climate. Rainfall is heavy in both areas but greater in Nimboran where the seventeen-year average for January, February and March, the months of maximum precipitation, is 956 mm. as compared to 719 mm. in Sentani; and for June, July and August, the months of lightest rainfall, 417 mm. and 281 mm. respectively.

Records of temperature and relative humidity made by the Sentani Meteorological Station for 1954 are reproduced here. They do not differ substantially from those of the other years.

	Temperature in °C.			Relative humidity (%)		
Hours	06.30	12.30	18.30	06.30	12.30	18.30
Yearly average	22.6°	30.3°	26.8°	95	65	80

July had the lowest temperature (21.9°C.), October the highest (31.3°C.). The absolute maximum and minimum were 34.5°C. and 17.8°C. respectively.

Inhabitants. The inhabitants of New Guinea are Papuans, a Melanesian negroid race. In the Sentani basin the native villages, inhabited by some 5,500 Papuans, are mainly situated in the lake area, some on small islands, others along the shore with houses built over the water. There are also 5 inland villages in the area along the western part of the road with about 1500 Papuans and settlers, and on the plains north of the lake a few hundred people live in their gardens. Mosquito nets are seldom or carelessly used. More than 400 Sentani people, mainly from the eastern lake villages, work and live in Hollandia, and their relatives, coming to the capital for business or amusement, frequently spend the night there.

Three languages are spoken in the region, one in the inland villages, one in the lake villages and the third in the Nimboran, the inhabitants of which have little or no contact with the Sentani.

Houses. The native houses are built on piles and both the inner and outer walls are made of "gaba gaba", or ribs of the sago-palm fitted together tightly and set vertically between the beams of the wooden frame; the roofs are of "atap", made from palm leaves. Another species of palm provides the floor planks. Corrugated iron is also used for the huts.

THE EXPERIMENT

Every inhabitant of the lake or experimental area, and the children in the control area, were registered. The date of birth of many of the children was obtained from the Mission register and the age of the others estimated.

Spleen and parasite surveys. Spleens were examined and thick Giemsa-stained blood films made of a representative sample of the people in the lake area before spraying, first in June, 1953; a second time between October and December, 1953; while in January, February and March, 1954, the children were again examined for malaria on the day their village was to be sprayed. After the spraying the infants were regularly examined for malaria at intervals of 1 or 2 months. One year after the first application of insecticides a complete survey of all ages was made (Table 1).

Surveys were made in the 5 native villages of the surrounding area before the spraying in February, 1954, and the infants were examined on 4 occasions after spraying.

In the control area complete surveys were made in May, 1953; February, 1954; July, 1954; and July, 1955; while surveys of infants were made in October, 1954, and in February and May, 1955. This area has now become an experimental area for a new project.

Spleens are always palpated with the subject in a recumbent position and recorded according to Schüffner's method. Only thick Giemsa-stained blood films are made. Infections are described as heavy when every microscopic field is positive, indicating 500 or more parasites per cmm. of blood.

Entomological surveys. The small staff collected as much entomological data as possible, which increased as more personnel was taken on and became better trained. Before the spraying in 1953, mosquitoes were collected only in Dojo, one of the western hyperendemic lake villages. In 1954, after the spraying was begun, catches were made at regular intervals throughout both the experimental and control areas at approximately the same time. In 1955 collections were made much more frequently in the Nimboran than in the lake area in order to study the bionomics of the local mosquitoes.

The mosquitoes were caught by day indoors from the house walls and at night out of doors on human bait. They were dissected according to Sergent's method by cutting off the head and pressing out part of the contents of the thorax on the slide, which was then stained with Giemsa and examined in the malaria service laboratory at Hollandia-Binnen. In June, 1955, gland dissections replaced Sergent's method.

Spraying. The lake and surrounding area were first sprayed between January 4th and April 9th, 1954, with a suspension of 5 per cent DDT wettable powder, sometimes replaced in non-native houses by 5 per cent DDT in kerosene. An average of 2.4 grams was used to make sure that about 2 grams of technical DDT was applied per square meter. Successive sprayings are done at intervals of 6 months. So far, 4 sprayings have been completed. The Lofstrand sprayer, type 250 AP., fitted with S.S. 8002 stainless steel teejet tip, renders good service. The walls are sprayed completely; the under-surface of the roofs only near the eave-

TABLE 1

Malaria indexes before and one year after spraying the hyperendemic and mesoendemic areas, compared with the control area

	Spleens								Parasites						
	Infants		Above 1 year						Infants		Above 1 year				
	0-6 mos.	7-12 mos.	1-5 yrs.	6-14 yrs.	15-44 yrs.	45+ yrs.	Totals		0-6 mos.	7-12 mos.	1-5 yrs.	6-14 yrs.	15-44 yrs.	45+ yrs.	Totals
1. Hyperendemic lake area															
Before spraying								Before spraying							
No. exam'd	39	28	336	408	656	128	1595	No. exam'd	39	28	336	387	649	127	1566
Spl. index	46	67	92	99	86	82	80%	Par. index	28	70	79	49	28	32	47%
Av. spleen	1.0	1.5	2.4	2.5	1.7	1.7	2.0	% heavy infect	27	33	39	4	0.7	0	17
One year after spraying								One year after spraying							
No. exam'd	49	33	364	403	375	77	1301	No. exam'd	49	33	363	403	376	77	1301
Spl. index	54	53	67	98	86	86	74%	Par. index	0	7	43	37	14	13	20%
Av. spleen	0.6	0.6	1.3	2.0	1.6	1.6	1.7	% heavy infect's	0	7	15	2.6	0.7	0	6
2. Mesoendemic lake area															
Before spraying								Before spraying							
No. exam'd	48	46	478	661	817	125	2175	No. exam'd	48	45	478	654	813	124	2162
Spl. index	39	56	38	52	57	50	48%	Par. index	12	7	20	22	13	16	16%
Av. spleen	0.5	0.6	0.5	0.8	0.9	0.8	0.7	% heavy infect's	0	2.5	4	5	2	0	3
One year after spraying								One year after spraying							
No. exam'd	71	52	311	579	265	64	1342	No. exam'd	71	52	311	580	277	66	1350
Spl. index	52	43	23	46	66	64	49%	Par. index	1	4	7.6	10	7	5	6
Av. spleen	0.6	0.6	0.3	0.6	0.9	0.9	0.6	% heavy infect's	0	0	0.5	1.0	0.3	0	1
3. Nimboran control area															
Survey, Feb. 1954								Survey, Feb. 1954							
No. exam'd	26	23	204	249	478	26	1006	No. exam'd	39	27	182	238	232	52	1001
Spl. index	57	97	96	97	88	70	84	Par. index	79	70	96	99	93	83	87%
Av. spleen	1.2	3.2	3.0	2.3	1.8	1.5	2.2	% heavy infect's	1.6	3.3	2.8	2.4	1.8	1.4	2
Survey, July 1955								Survey, July 1955							
No. exam'd	26	23	203	248	475	26	770	No. exam'd	40	29	182	236	233	53	773
Spl. index	64	97	88	71	39	23	64	Par. index	52	88	91	73	42	30	64%
Av. spleen	2.0	3.3	2.8	2.4	1.8	1.7	2.3	% heavy infect's	17	59	60	12	1.7	2	25

openings. Special care is taken that the floor-wall junction gets enough insecticide. Galleries are included, likewise the sparse animal sheds. As a rule the underside of the houses is not sprayed. Whether furniture is sprayed or not depends on the quality; usually the natives do not object to it. The cooperation of the people is excellent, largely because they were instructed beforehand with the help of photographic slides and a keroscope projector.

MALARIA TRANSMISSION BEFORE SPRAYING

Morbidity and mortality. The available figures on malaria morbidity and mortality do not for various reasons represent the actual situation. Drugs are easily obtainable at dispensaries and hospitals in both the Sentani and the comparison areas, and moreover a motor launch of the Government Health Service visits lake villages twice a week; but treatment is not sought with regularity and has probably little influence on malaria morbidity, an impression confirmed by the surveys made before the insecticide spraying was done.

Spleen and parasite indexes. No significant seasonal influence was observed in this or previous surveys. As noted by Swellengrebel and Swellengrebel (1948), and by Swellengrebel (1949), malaria transmission was more intense in the western part of the lake district where the villages lie near the lake shore, than in the eastern part where all but two villages are situated on the long Ajapo peninsula or on small islands whose steep slopes provide no opportunities for mosquito breeding. The few anophelines captured there must have come from the lake shore, for the greater the distance from shore, the fewer were caught during a ten-day observation period on three of the islands. It is evident that the islanders are infected only when they spend the night ashore. The malaria figures from the two eastern villages on the lake shore are similar to those of the western part.

The intensity of transmission in the hyperendemic lake area is revealed by the parasite indexes of the infants, who become infected in the early months of life and are 70 per cent infected at 6 months (Table 1). At the end of the first year all are infected, so that the conditions approach those of holoendemicity (Wilson *et al.*, 1950). Group immunity develops fairly rapidly as shown by the significant fall in the parasite index with advancing age, apparent also though less marked in the spleen index and average spleen size (Table 1). The comparison area, Nimboran, presents the same picture of hyperendemic malaria. A survey of the less malarious eastern area showed a much lower infection rate, with little indication of acquired immunity in the spleen indexes or average size of spleen. The parasite index, however, diminished significantly after the age of 15 (Table 1).

ENTOMOLOGICAL DATA

Treated area. Before spraying, the following anopheline species were dissected in Dojo, in the hyperendemic lake area: *A. punctulatus*, 4; *A. farauti*, 261 (1 positive); *A. koliensis*, 348 (4 positive); *A. karwari*,[2] 128 (4 positive); and *A. longirostris*, 3. Thus there were 9 positives (1.2 per cent) among the 744 mosquitoes

[2] Incriminated in 1953 as a vector in New Guinea by Metselaar (1955).

dissected, of which 182 were collected indoors by day, i. e., *A. farauti* 13; *A. koliensis* 128 (2 positive) and *A. karwari* 41 (2 positive).

After spraying, mosquito catches were continued in Dojo, when 94 *A. punctulatus*, 437 *A. farauti* (2 positive), 719 *A. koliensis* (2 positive), 406 *A. karwari* and one *A. bancrofti* were caught and dissected, yielding 4 positives (0.2 per cent) in 1657 dissections. Only one anopheline was caught indoors and that in a newly built and unsprayed house.

Ten days were devoted to catching mosquitoes on three of the mesoendemic islands, but because of strong evening winds the only catches made were indoors by day when 159 were collected from the walls: *A. punctulatus*, 9; *A. farauti*, 13 and *A. koliensis*, 137. On Jobe, the island nearest the shore, 101 were collected: *A. punctulatus*, 4; *A. farauti*, 9; *A. koliensis*, 88. These were the only mosquitoes dissected and all were negative.

Control area. In January, 1954, dissections of mosquitoes were begun in the control area, using Sergent's method, and by February, 1955, 3,078 had been done: *A. punctulatus*, 2453 (25 with sporozoites); *A. farauti*, 199 (2 with sporozoites); *A. koliensis*, 413 (2 with sporozoites); and *A. karwari*, 13. In all there were 29 infected mosquitoes (nearly 1 per cent). Later, the glands of 694 mosquitoes were dissected and 10 (1.5 per cent) were found to be positive as follows: *A. punctulatus*, 296 (4 positive); *A. farauti*, 126 (3 positive); *A. koliensis*, 268 (3 positive) and *A. longirostris*, 4. Of these 3,772 dissected anophelines from the Nimboran, 2,181, of which 27 were infected, were collected indoors by day.

From January 18 to July, 1955, catches were made on 81 nights; two laboratory assistants caught mosquitoes from sunset till midnight, two others from midnight till dawn. These men collected only the mosquitoes which attacked their own legs when they imitated the local people, sitting outside the house or on the gallery when the people did, and then catching indoors when they went inside to sleep. They collected 34 mosquitoes per person per night, with an infection rate of one to one-and-one-half per cent, which means 125 to 180 bites by infected mosquitoes per unprotected person per year.

MALARIOLOGICAL DATA ONE YEAR AFTER SPRAYING

Table 1 and Figures 1 and 2 give the results of the survey in the hyperendemic lake area made one year after the first spraying. These show that infants born after the spraying remained almost free of infection; the parasite rates of the other age groups had dropped considerably; the infections that occurred were less heavy, and fewer were mixed infections. The spleen rate, perhaps due to fibrosis, was only slightly reduced: the average enlarged spleen from 2.3 to 2.0 and the average spleen from 2.0 to 1.7. The change in the parasite formula is indicative of the general improvement:

	1953 Oct.-Dec.	1955 Jan.-Mar.
P. falciparum	43%	27.5%
P. vivax	22%	16.0%
P. malariae	35%	56.5%

The improvement in the mesoendemic lake area was even greater; the parasite rate was much lower, and the parasite ratios considerably changed:

	1953 Oct.–Dec.	1955 Jan.–Mar.
P. falciparum	50%	27%
P. vivax	22%	38%
P. malariae	28%	35%

There was little change in the spleen rate. In July, 1955, 8 of 123 infants from the hyperendemic lake area were positive and only one of 174 from the mesoendemic lake area, while before spraying about 85 would have been infected in the two areas. Of the nine infections in 1955, three were probably infected while staying in unsprayed Hollandia and one infection may have been congenital.

In the December, 1955, survey of the 5 native inland villages, only 2 of 13 infants were infected, one of whom had been to the unsprayed Grimé Valley; before spraying, 14 out of 15 infants had been found infected.

The high number of palpable spleens in malaria-free infants in both lake areas is striking. Up to the age of 7 months the spleen rate of these babies did not differ

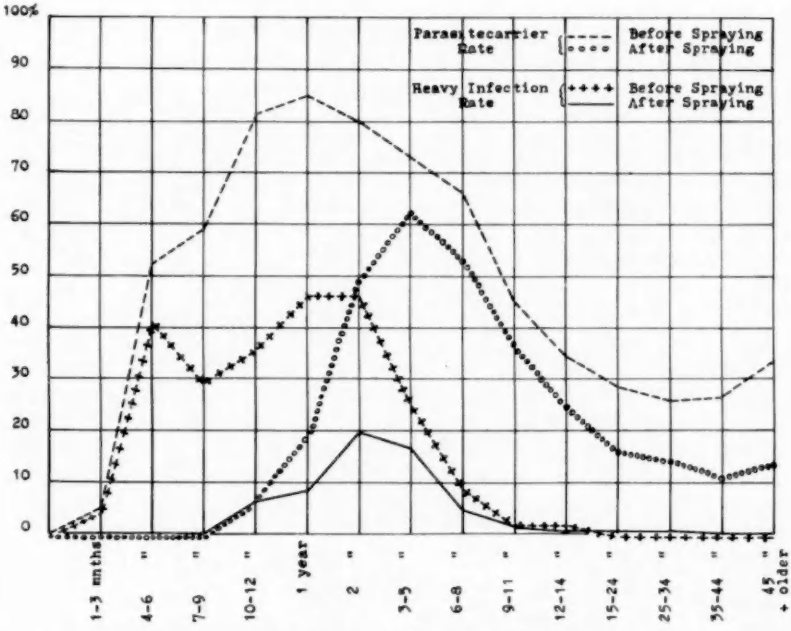

FIG. 1. Parasite carrier rates and heavy infection rates of the hyperendemic lake area, before and one year after spraying.

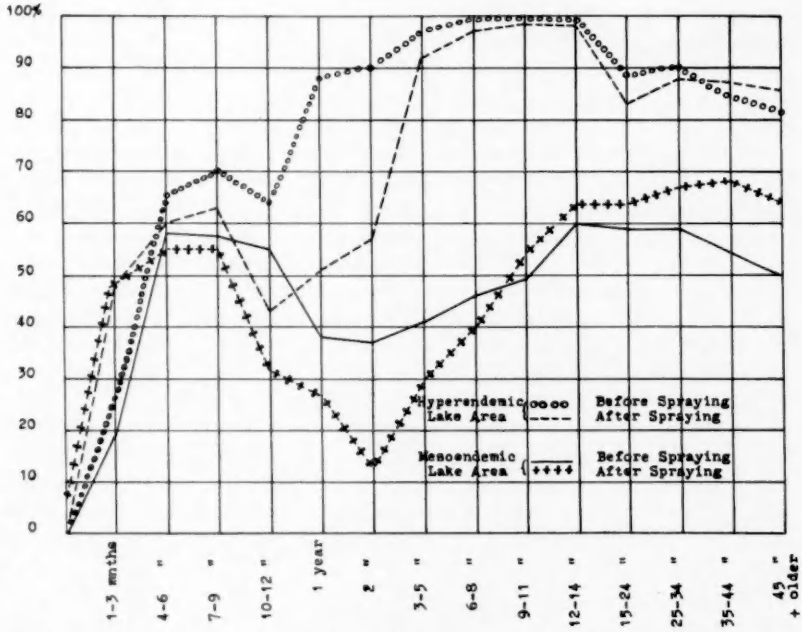

FIG. 2. Spleen rates of hyperendemic and mesoendemic lake areas, before and one year after spraying.

from that recorded before spraying. The peak of 68 per cent was reached at seven months after which it began to go down, but at 16 months the spleen rate was still 33 per cent. This phenomenon is still under investigation and will be discussed elsewhere.

Table 1 gives the results of the July, 1955, survey of the control area which differ little from the February, 1954, survey. The age groups are not represented equally in the various surveys so the totals in the tables have only a relative value for comparison.

DISCUSSION AND SUMMARY

Until recently the basin of Sentani Lake and the Grimé Valley which are representative of many parts of Netherlands New Guinea and also of areas of Australian New Guinea (Avery, 1946; Bern and Hansen, 1950; Hairston *et al.*, 1947; Mackerras and Aberdeen, 1946) were both highly malarious due to the presence of potent vectors of the *Anopheles punctulatus* group and also, in the Sentani area, of *A. kawari*.

In 1954, the houses of the Sentani area comprising the villages on and around the lake where observations were made, and a surrounding area where the villagers have their gardens, were sprayed with DDT, leaving the Nimboran, the

western part of the Grimé Valley, as control since surveys had shown malaria to be hyperendemic in both valleys.

The spraying did not completely prevent transmission as a few sporozoite-positive mosquitoes were found and a few new infections occurred during the following year in infants who had not been outside the sprayed area, amounting however to less than $^1/_{10}$ the expected number before spraying. Surveys made one year after the spraying showed considerable improvement in the malaria situation, with a much lower parasite rate, which dropped from 46 to 32 per cent in the hyperendemic lake area and from 18 to 8 per cent in the mesoendemic lake area, while that of the control area remained approximately the same. In the sprayed area the sporozoite rate in the *Anopheles* dropped from 1.2 to 0.2 per cent. Improvements in the spleen rates were less marked, due perhaps to splenic fibrosis. Also the high spleen rate in malaria-free infants is striking and should be investigated, though palpable spleens in infants need not be of pathological significance.

Since February, 1955, the houses in a large territory around the experimental area, including Hollandia and the Grimé Valley, have been sprayed so that the risk of infection both inside and outside the Sentani basin is now equalized and minimized. Eradication of the local vectors and of the malaria parasites in New Guinea cannot be anticipated by residual spraying, but a much lower degree of endemicity seems reasonable to expect.

ACKNOWLEDGEMENT

I wish to thank Dr. I. C. Fang, Director, and Dr. F. J. Dy, Malaria Adviser of the Regional Office of W.H.O. at Manila, for the DDT which they kindly provided.

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SOME ASPECTS OF CONCOMITANT INFECTIONS OF PLASMODIA AND SCHISTOSOMES. I. THE EFFECT OF *SCHISTOSOMA MANSONI* ON THE COURSE OF INFECTION OF *PLASMODIUM BERGHEI* IN THE FIELD VOLE (*MICROTUS GUENTHERI*)

MEIR YOELI

Department of Parasitology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Concomitant infections of malaria and schistosomiasis have frequently been observed in areas where both diseases are endemic. The association of these two infections and their chronic course, the appearance in cells of the reticulo-endothelial system of an iron-containing, hemoglobin-derived pigment, have, among other factors, induced some authors in the past to attribute the distinct pathological syndrome of liver cirrhosis and Egyptian splenomegaly to bilharzial or malarial origin or to their combined effect. Although the course of infection and the pathology of each of these diseases has been studied in detail, both in man and in laboratory animals, no experimental work has hitherto been described on the effect of combined infections of malaria and schistosomiasis in suitable experimental animals and on the influence of one parasite on the other. The discovery of *Plasmodium berghei* by Vincke and Lips (1948) has very much facilitated such an experimental approach, owing to the wide range of rodent hosts susceptible to both infections.

The present paper is part of a study of concomitant infections in different laboratory animals.

METHODS

The strain of *S. mansoni* was originally isolated by Professor G. Witenberg in 1952 from a patient who immigrated from the Yemen, Arabia. The strain has been kept in *Australorbis glabratus* and golden hamsters were routinely infected by exposure to cercariae according to the immersion method of Standen (1949). As eggs of this strain of *S. mansoni* are very rarely passed in the feces of infected hamsters, all eggs for miracidia hatching and snail infections were obtained from homogenized infected liver tissue. Field voles *Microtus guentheri* were received from a colony of a local strain bred and maintained in the animal house of the Hebrew University. All the infections with *S. mansoni* were carried out by the percutaneous route with a standard dose of 100 pooled cercariae, obtained from several snails in order to ensure a bisexual infection. Animals were killed at intervals and pieces of tissue from liver, spleen, lungs and intestine were taken. They were fixed in Zenker and stained in hematoxylin-eosin. Macroscopic examinations were carried out in all sacrificed animals. Adult worm counts were made in 12 infected voles. Fecal specimens from all infected animals were obtained twice weekly and examined for eggs of *S. mansoni* by simple smear and sedimentation method (Tomb and Helmy, 1931). The strain of *Plasmodium berghei* (Kassapa III) was received by the courtesy of Professor J. Rodhain from the

Prince Leopold Institute, Antwerp. It was derived from sporozoites of an infected *Anopheles dureni*. The behavior of this strain and its pathogenicity for experimental animals was found to be very similar to the old strain of *P. berghei*, which was obtained in 1950 from the London School of Hygiene and Tropical Medicine and which was used in the work of Zuckerman and Yoeli (1951, 1954). The animals were infected with a measured amount of 40 million parasitised erythrocytes and blood films were taken daily during the patent period and twice weekly during the post-patent period. The intensity of the plasmodial infection was expressed as the number of parasitised erythrocytes per 10,000 examined in stained smears from the peripheral blood.

THE COURSE OF INFECTION AND PATHOLOGY OF *S. MANSONI* IN *M. GUENTHERI*

In order to study the effect of *S. mansoni* infection in the field vole 18 *M. guentheri* were infected in groups of four, each animal received 100 cercariae. The animals were sacrificed at various intervals and a histological study of their organs, especially of the liver was carried out. The number of voles used and the duration of their infection are summarized below.

Number of Animals	Period of Observation After Exposure to Cercariae of *S. mansoni*
1	1 week
1	2 weeks
3	4 weeks
2	6 weeks
2	8 weeks
4	12 weeks
1	14 weeks
2	16 weeks
1	20 weeks
1	22 weeks
Total..... 18	

In addition to the 18 animals which were sacrificed during various stages of their infection observations were carried out on 6 voles in which the infection was allowed to run a natural course. Of them one died after $7\frac{1}{2}$ months, four lived for 9 months and were then autopsied and one was accidentally killed 328 days after exposure to cercariae. Egg depositions and macroscopical changes in the livers were found in all of them on post mortem examination. Histological sections from nine uninfected control animals were also prepared in order to distinguish between early histopathological changes in the liver caused by *S. mansoni* and non-specific cellular reactions encountered in apparently normal animals.

RESULTS

In three of the livers from the 9 uninfected voles, small areas of lymphocytic, mononuclear and polymorphonuclear infiltrations were found around portal

venules. They resembled in general the cellular infiltrations in uninfected white mice as described by Meleney *et al.* (1952). In two of the controls small circumscribed areas of infiltration in the liver parenchyma were also observed.

The gross pathology and the histopathological picture of the infected *M. guentheri* varied with the period following exposure to cercariae. In the first two weeks no marked pathological changes could be detected. After four weeks, very little could be seen macroscopically, but on histological examination small scattered areas of fatty degeneration and hepatitis were seen. The monocytic and polymorphonuclear infiltration near and around portal vessels was increased. No enlargement of spleen was seen at this period. In one out of three voles examined at this stage, an area of coagulative necrosis was found in the parenchyma of the liver. After six weeks when eggs first appear in the liver, the pathological changes were to a large degree connected with tissue reactions around the deposited eggs. There were different grades of reaction. In a few cases when eggs were found lying in portal tracts, a very mild reaction ensued with a single layer of epithelioid cells encompassing the egg. Pseudotubercle formations were found in 8 out of 13 animals examined between the 7th and 22nd week after infection (Fig. 1). The egg was usually surrounded by fine fibrous tissue, small round cells, plasma cells, epithelioid cells and eosinophil leucocytes (Fig. 2). When several eggs were deposited in portal connective tissue a localised fibrotic reaction took place (Fig. 5, 6). Although in a few cases there was a tendency to fibroblastic proliferation between adjacent or topographically remote eggs lying single or in groups no generalised fibrosis was seen in any of the livers of the 18

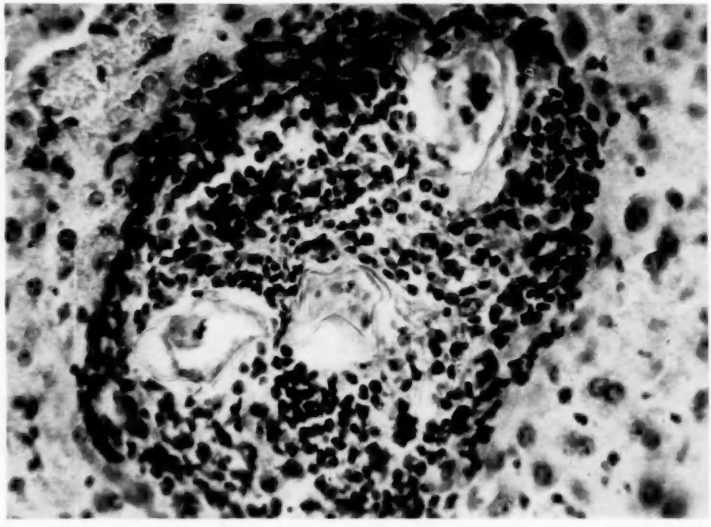

FIG. 1. Liver of *M. guentheri* infected with *S. mansoni* showing pseudotubercle containing three eggs. × 360

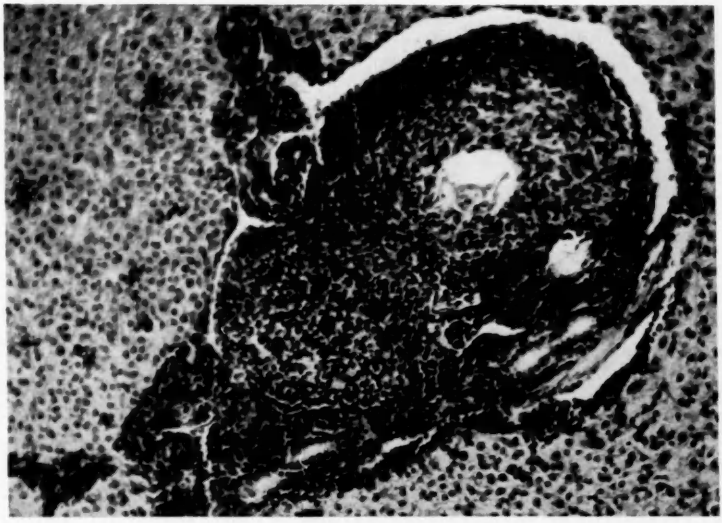

FIG. 2. Liver of *M. guentheri* infected with *S. mansoni* showing extensive infiltration around egg involving biliary ducts. × 150

M. guentheri infected with the above strain of *S. mansoni*. Together with the cellular reaction around the deposited eggs, areas of coagulative necrosis, as described by Meleney were seen (Fig. 3). In these well demarcated areas varying in size, no signs of egg shells or of adult worms were found and their appearance may be interpreted as a direct toxic effect on the liver parenchyma. In 5 of the 18 livers examined bilharziomata (Gelfand, 1950) containing a large number of eggs were seen. Giant cells were not only found in tubercles but also scattered throughout the lobules. These giant cells contained a large amount of golden-brown pigment (Fig. 4).

Egg passage. Fecal examination for eggs of *S. mansoni* was carried out in all infected animals. The examinations commenced 4 weeks after exposure to cercariae, at half week intervals up to 26 weeks. No eggs were found except in one single case in which a few were seen in the stool of a vole whose infection was of 10 weeks duration. On the other hand, heavy viable egg concentrations were found in the liver and intestines. They were easily detected macroscopically in press preparation and later in histological sections. Most of the eggs were viable as confirmed by miracidia hatching and subsequent snail infection. No eggs were found in the lungs of the voles examined, and the spleen only rarely harbored a few. From the above observations it appears that hamsters and voles although readily infected with this Yemenite strain of *S. mansoni* do not excrete eggs with their feces and could not therefore serve as natural carriers for cyclical transmission.

Recovery of worms. Twelve *M. guentheri* sacrificed between 8 and 14 weeks

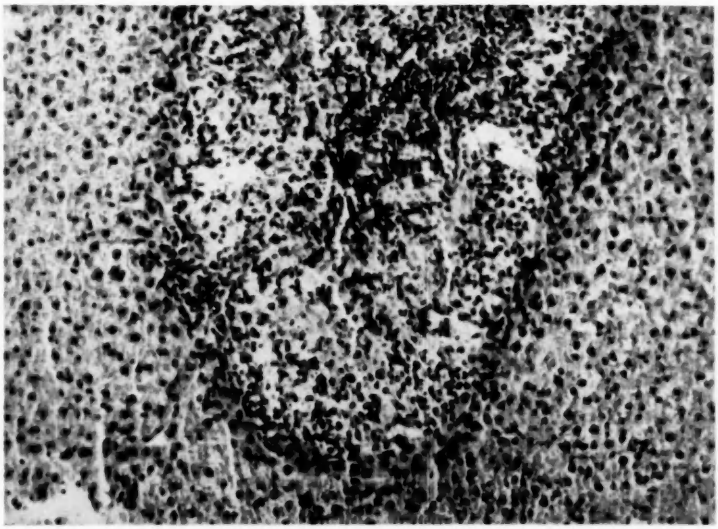

FIG. 3. Liver of *M. guentheri* infected with *S. mansoni* showing area of coagulative necrosis and cellular demarcation. 10 weeks after exposure to cercariae. × 150

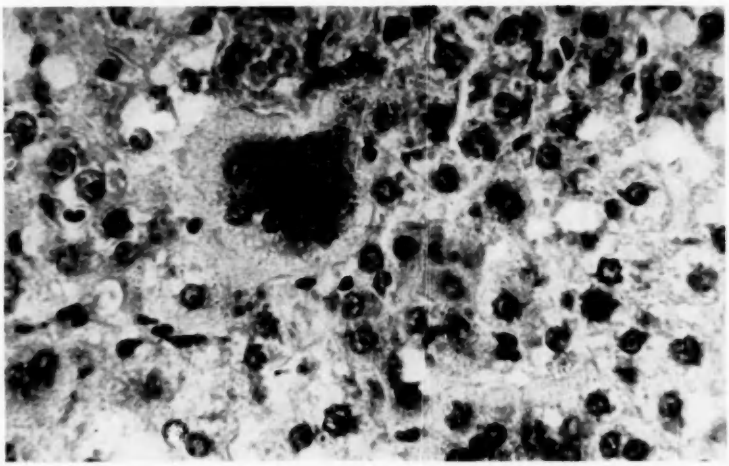

FIG. 4. Giant cells containing brownish-golden pigment among parenchyma cells of the liver. From a mixed infection of *S. mansoni* and *P. berghei*. × 560

after exposure to 100 cercariae were examined for adult worms. The pressed liver tissue, the intrahepatic and mesenteric part of the portal circulation were searched, the worms removed and counted. The numbers found are given in Table 1. The average recovery was 13 per cent, the lowest number of worms found was 6, the highest 19.

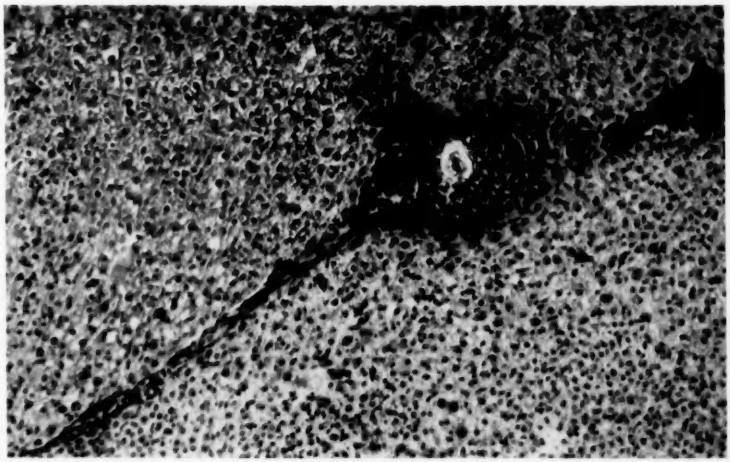

FIG. 5

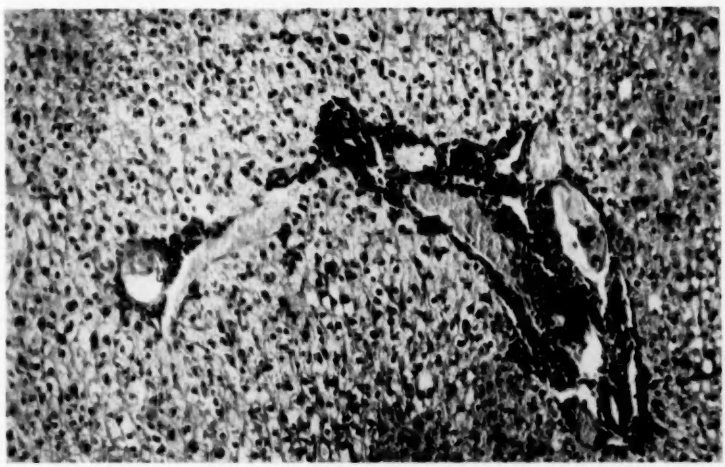

FIG. 6

FIG. 5, 6. Liver of *M. guentheri* infected with *S. mansoni* showing localized fibroblastic proliferation between adjacent groups of eggs. × 120

Eosinophilic reaction. In seven of the infected *M. guentheri* differential counts were made twice weekly for a period of 23 weeks. In all the animals examined there was a marked rise in the eosinophiles reaching a peak of 24 per cent at the end of the fourth or in the fifth week after cercaria penetration. During the 6th and 7th week the percentage of eosinophiles fluctuated between 19 and 12 per cent. Their number diminished markedly in the peripheral blood after the 7th week, averaging 3–5 per cent during the next 14 weeks.

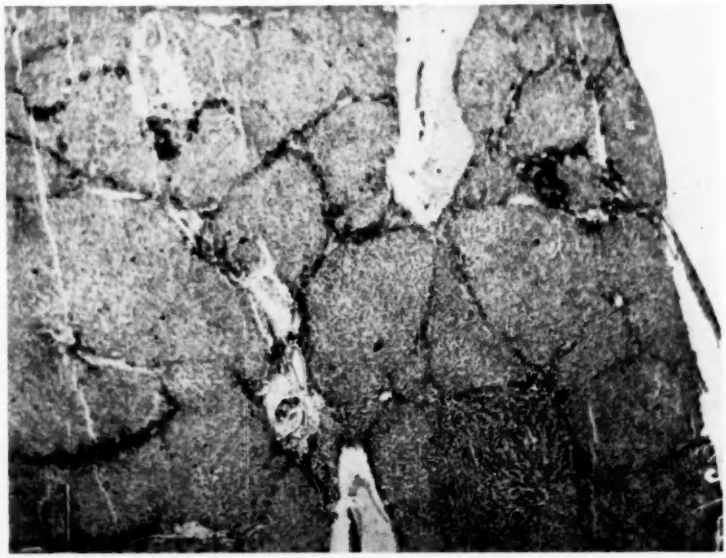

FIG. 7. Liver of *M. guentheri* (N .281) infected with *S. mansoni* and *P. berghei* showing generalized cirrhosis (4 weeks after exposure to cercariae). × 33

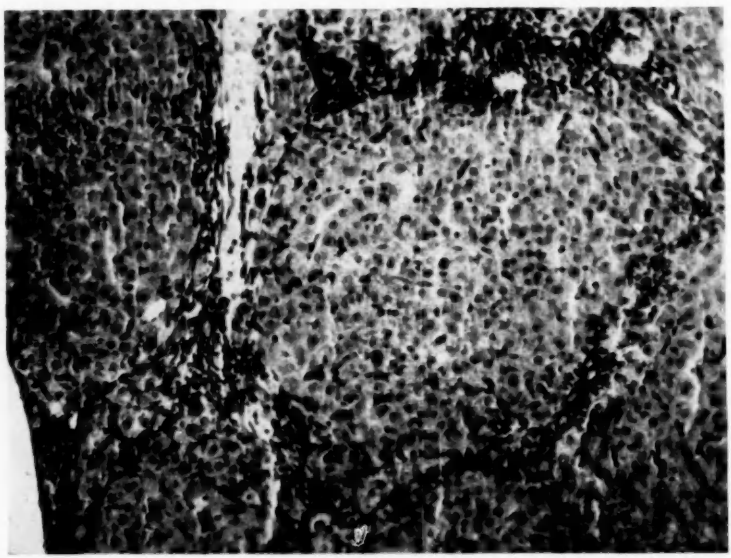

FIG. 8. Same section as Fig. 7 seen in high magnification, showing a ring of connective tissue compressing a lobule. × 150

TABLE 1

Recovery of S. mansoni *worms from 12* M. guentheri *each exposed to 100 pooled cercariae from several snails*

Number of Animals	Weeks After Exposure	Average Number of Worsm Recovered	%
3	8–9	10	10
4	10	13	13
4	12	16	16
1	14	13	13

The course of infection of P. berghei *in* M. guentheri. The course of infection of *P. berghei* in the field vole *M. guentheri* was studied in 46 animals. All were inoculated with a standard dose of 40 million parasitized mouse red cells. Parasites were found in the peripheral blood early after a prepatent period of 2–3 days. The plasmodial infection ran a mild course in 36 of the 46 animals (78.2 per cent) terminating in latency or in spontaneous radical cure after a patent period lasting between 4 and 15 days (average patency period—9 days). Parasites reached their peak on the 7th day after infection but their number at the height of parasitemia was usually low, varying in the different animals between 3 and 7 per cent. Low grade spontaneous relapses were observed in a number of animals. They occurred early in latency, between the 6th and the 14th day following its onset.

Ten *M. guentheri* of the group of 46 animals succumbed to their plasmodial infection (mortality 21.7 per cent). The intensity of parasitemia in the peripheral blood at death was 70–80 per cent. Of the surviving 36 animals, 27 were observed for a further period of seven weeks. Blood examinations carried out three times weekly, failed to reveal any parasites in the peripheral blood during the whole period of observation.

A complete recovery was observed in a single *M. guentheri* who developed a fulminant infection with a peak parasitemia of 67 per cent on the 9th day. In this animal parasites disappeared from the blood within 48 hours. The occurrence of spontaneous radical cures in voles, survivers of untreated infections, as shown in an earlier work by Zuckerman and Yoeli has been confirmed in animals of this group. Injection of the total volume of cardiac blood from 9 *M. guentheri*, four weeks after their initial plasmodial inoculation failed to produce *P. berghei* infection in susceptible mice.

Mixed infections of P. berghei *and* S. mansoni. Thirty *M. guentheri* were used in these experiments. Of these 5 were infected firstly with *P. berghei* and subsequently after 1 to 2 weeks with *S. mansoni*. The other 25 were inoculated with *P. berghei* at various intervals from 5 days to 48 days after exposure to cercariae. All animals of this group were sacrificed at different stages of their plasmodial infection and touch preparations as well as histological sections from liver, spleen, lungs and intestines were prepared. The number of animals, the period of their exposure to *S. mansoni* and *P. berghei* and the outcome of their plasmodial infection are summarized in Table 2.

TABLE 2

Resumé of data showing behaviour of P. berghei *in 30* M. guentheri *infected with* S. mansoni *at different periods of their plasmodial infection*

Microtus Number	Infection *P. berghei* Days (−) Before or (+) After *S. mansoni*	Days Patent Before Exposure to *S. mansoni*	Days Patent After Exposure to *S. mansoni*	Total Days of Observation After *P. berghei*	Final Outcome of *P. berghei* Infecton
274	−7	3	38	45	Chronic*
275	−7	4	9	16	Death
276	−7	3	4	42	Recovered†
277	−14	5	35	49	Chronic
278	−14	3	8	22	Death
279	+5	—	2	72	Recovered
280	+7	—	1	83	Recovered
281	+7	—	24	24	Death
282	+7	—	3	67	Recovered
283	+7	—	65	67	Chronic
284	+14	—	6	96	Recovered
285	+14	—	39	40	Chronic
286	+14	—	32	34	Chronic
287	+14	—	10	12	Death
307	+16	—	3	10	Recovered
308	+16	—	45	46	Chronic
291	+28	—	0	64	Recovered
292	+28	—	2	82	Recovered
293	+30	—	21	24	Chronic
294	+30	—	3	65	Recovered
295	+30	—	3	83	Recovered
296	+30	—	0	80	Recovered
297	+36	—	2	108	Recovered
298	+36	—	31	34	Chronic
299	+38	—	0	96	Recovered
300	+38	—	0	72	Recovered
301	+42	—	0	112	Recovered
302	+42	—	4	94	Recovered
304	+40	—	3	80	Recovered
306	+48	—	2	82	Recovered

* Chronic—parasites present in blood smears taken three times weekly during whole period of observation.

† Recovered—no parasites found in blood smears.

The experimental data given in Table 2 show that 2 out of 5 voles infected with *P. berghei* one to two weeks before exposure to *S. mansoni* developed chronic infections exceeding 35 days of primary patency; two animals died of their plasmodial infection 16 and 22 days after inoculation with *P. berghei* and only one vole after an initial patency of 4 days showed a persistent latency of 42 days with no microscopically detectable parasites in the blood.

The second group consisting of 11 animals received their *P. berghei* infection 1 to 2 weeks after exposure to *S. mansoni*. In this group, 2 voles died of their plasmodial infection, four developed a definite chronic course with patency period lasting between 34 and 67 days respectively. Five animals after initial

short, low grade parasitemia did not show any parasites for the whole subsequent period of observation.

The third group of 14 *M. guentheri* received their plasmodial infection at periods ranging from 4 to 7 weeks after exposure to *S. mansoni* cercariae. In this group no deaths from *P. berghei* infection occurred. Two animals showed a chronic course of infection (14 per cent). In 5 of the 12 animals no initial microscopically detectable infection was seen at any time during the whole period of observation.

Effect of superinfection. Six *M. guentheri* from the group which received its plasmodial inoculation 4 to 7 weeks after *S. mansoni* infection and subsequently developed either a prolonged latency or a spontaneous cure, were inoculated with 850 million *P. berghei* parasites each intraperitoneally. In four of this group blood smears remained negative for a period of 46 days subsequent to superinfection; in one animal a mild (0.3 per cent) plasmodial infection developed for 72 hours only; one animal showed a 0.2 per cent infection which lasted 48 hours.

Pathology of the liver in mixed P. berghei *and* S. mansoni *infections.* In infected voles sacrificed during the patent period of their plasmodial infection a similar picture to that noted in rats infected with *P. berghei* and in other mammalian malaria was seen: hemozoin-containing swollen Kupffer cells in mid-zone areas, varying in intensity according to the severity of parasitemia. No fatty degeneration was seen in animals sacrificed during their patent period.

A comparative study of livers from *S. mansoni* infections only and those of mixed *P. berghei* and *S. mansoni* infections did not reveal evidence of enhanced pathological changes in the later groups in all those animals in which the plasmodial infection had a mild course and terminated in latency or cure. In two voles (298 and 308) in which *P. berghei* developed a chronic course, evidence of increased connective tissue reaction showing no topographical association with schistosomal eggs or worms was noted.

A characteristic, generalized early liver cirrhosis was seen in one *M. guentheri* (No. 281) which succumbed to *P. berghei*, twenty-four days after inoculation. This animal had received 40 million infected erythrocytes 7 days after exposure to 100 cercariae. It showed a peak parasitemia of 32 per cent. On macroscopic examination the liver was small, grey and shrunken, with fine nodules. As was to be expected, no *S. mansoni* eggs were present in this early one-month-old bilharzial infection. The histological picture as seen in hematoxylin and special connective-tissue-stained sections, was of a generalized cirrhosis. *P. berghei* parasites were found in large numbers in vessels and engulfed in Kupffer cells. A single immature worm was found in the liver section.

The finding of a distinct cirrhosis of the liver at such an early stage of schistosomal infection in an experimental animal raises the problem of the importance of concomitant diseases as factors enhancing generalized liver cirrhosis in man. Since the above findings are confirmed to a single animal obviously no definite conclusion can be drawn. Nevertheless they are of some interest in view of the fact that the general pathology of *S. mansoni* infections is very similar in white mice and voles (Moore *et al.*, 1949). Meleney found no signs of a generalized

fibrosis at any stage of the infection in white mice among very extensive material examined.

DISCUSSION

In the experiments reported of mixed *P. berghei* and *S. mansoni* infections a marked increase in chronic plasmodial infections was observed. They were conspicuous in the groups of animals which received their plasmodial infection 1 to 2 weeks before exposure to *S. mansoni* as well as in the group in which the malaria infection followed 1 to 2 weeks after exposure to cercariae. As is known, this period of the invasion and growth of schistosomules is not marked by tissue reaction, proliferation or infiltration. It has been stated that this primary invasive period is marked by its general toxic reaction. The chronic course in 6 out of 16 animals in this group (37.5 per cent) must be considered definitely as deviations from the normal course of *P. berghei* infections as seen in *M. guentheri* with plasmodial infections alone. It may therefore be presumed that concomitant infection with schistosomes developing in the same host deprives the vole in many cases of its ability to suppress or totally eliminate the plasmodial infection. The mechanism of this interference is unknown, but a similar phenomenon has been observed in splenectomized voles, developing long chronic infections.

In the third group of animals, e.g., those receiving their plasmodial infection 4 to 7 weeks after exposure to cercariae, no deaths occurred. The mild course of infection seen in this group in which 5 out of 14 animals did not show any parasites *ab initio* and 7 others only a very mild initial parasitemia followed by recovery or prolonged latency, coincides with a period of tissue reaction, proliferation and infiltration as a result of the concomitant bilharzial infection. It may therefore be presumed that an alerted reticuloendothelial system, with marked increase in the phagocytic power of the host organism due to bilharzial infections, markedly affects the course of a concomitant plasmodial infection.

SUMMARY

The development of *S. mansoni* from a strain derived from the Yemen (Arabia) has been followed in experimental infection in the field vole (*M. guentheri*). In this animal all the stages of schistosomal infection were noted and the histopathology of the liver in early and long standing infections observed.

The course of infection of *Plasmodium berghei* was studied in 46 *M. guentheri*. The mild parasitemias, the low mortality rate (21.7 per cent) and the absence of prolonged chronic infections were noted. The occurrence of spontaneous radical cures observed in earlier work has been confirmed.

The course of *P. berghei* infection in *M. guentheri* in mixed infections with *S. mansoni* depends on the stage of the bilharzial infection and probably on the cellular reaction of the host. *P. berghei* infections which preceded shortly, or followed 1 to 2 weeks after, exposure to *S. mansoni* showed a high proportion of infections with a chronic course. In a group of voles infected with *P. berghei* 4 to 7 weeks after *S. mansoni*, the plasmodial infection was distinguished by its

mild course, low parasitemia, no deaths from malaria and absence of parasites from the peripheral blood *ab initio* in 38.2 per cent.

A generalized liver cirrhosis at an early stage of bilharzial infection was observed in one *M. guentheri* with mixed *S. mansoni* and *P. berghei* infections.

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EXPERIMENTAL *ENTAMOEBA HISTOLYTICA* INFECTIONS IN MAN[1]

PAUL C. BEAVER, RODNEY C. JUNG, HARRY J. SHERMAN, THOMAS R. READ AND THOMAS A. ROBINSON[2]

Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, Louisiana, and Medical Department, Mississippi State Penitentiary, Parchman, Mississippi

Experimental infections in human volunteers have been reported by Walker and Sellards (1913), Simic (1931) and Westphal (1937). Collectively they provide observations on 25 infections. In connection with an experimental study of chemoprophylaxis of amebiasis involving more than a hundred human volunteers, recently reported (Beaver *et al.*, 1956), observations on other aspects of amebiasis were made. Those reported here fall into 5 categories: (1) susceptibility to infection and prepatency, (2) stability of infections, (3) susceptibility to reinfection, (4) symptomatology and pathogenicity in human volunteers and (5) pathogenicity in experimental animals.

MATERIALS AND METHODS

A description of the single strain ("H") of *Entamoeba histolytica* employed in the experiments, as well as a general account of the methods used in the inoculations and fecal examinations, have been published (Beaver *et al.*, loc. cit.). Suspended in carbonated beverage, 2,000 to 4,000 cysts, derived directly from a non-symptomatic carrier, or after passage through other volunteers, were given to 130 white and Negro male prisoners (including some who were used in more than one experiment) 19 to 55 years of age. Eighty-one infections resulted from inoculations that were given either as challenges to various chemoprophylactic regimens (33), as controls (34), or as preliminary trials of inoculation procedures (14).

RESULTS

1. *Susceptibility to infection and prepatency.* All of 42 individuals (14 in preliminary trials and 28 controls), not previously infected and not given drugs, became infected when given 2,000 to 4,000 cysts. Heavier inocula (10,000 to 1 million) produced infections in each of 4 others. Thus, all of these 46 individuals were susceptible to infection. Two of 3 individuals who were reinoculated after having spontaneously lost infections were susceptible, making a total of 48 infections which provided data on prepatency (Table 1). Stool examinations immediately following inoculation were made daily until positive on 14 individ-

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[2] Address Medical Dept., State Penitentiary, Parchman, Miss.

TABLE 1

Prepatent periods, if established, for 106 experimental Entamoeba histolytica *infections in human volunteers reported by various observers*

Days Prepatent	Walker & Sellards (1913)	Simic (1931)	Westphal (1937)	Present Study				
				Controls			Proph. Trials	Total
				d.c.	w.c.	h.i.		
1	2							2
2	2		1		1	2		6
3	1			1				2
4	4			2	2			8
5	3	1		3	14			21
6					4	1		5
7		3		1	2			6
8	1							1
9		1		2	1	1		5
11	2			1	2			5
12					4			4
14				2				2
21	1							1
26				1				1
126				1				1
Subtotal.....	16	5	1	14	30	4		70
Undetermined.....	2	1	—	—	—	—	33*	36
Total.....	18	6	1	14	30	4	33	106

d.c. and w.c.—Daily check and weekly check by fecal examination after inoculation of 2,000 to 4,000 cysts.

h.i.—Inoculation of 10,000 to 1 million cysts followed by irregular checks.

* Prepatency influenced by chemoprophylaxis (see Beaver *et al.*, 1956).

uals, 1 to 3 times weekly on the others. The shortest observed prepatent period was 2 days. It was 7 days or less in 33 instances; 9 to 14 days in 13, and 26 days in one. In one individual 51 stools were examined over a period of 112 days following inoculation without finding amebae (P-13 in Table 2). Saline purgation twice failed to produce recognizable amebae in the stools, but 3 weeks following the last purge the stools changed from green or pale brown, semi-formed, mucoid to more normal color and consistency, and from the 126th day to the end of the experiment cysts were abundant in each of 18 stools examined.

Of the 4 individuals given heavier inoculations of 10,000, 100,000, a half million and a million cysts respectively, the 2 given the least started passing cysts in 48 hours. The one given a million cysts submitted stools on the 3rd and 9th days; the latter was positive. The first stool submitted by the one given a half million cysts was positive 6 days after inoculation.

In summary, all of 46 experimental inoculations of 2,000 cysts or more administered to men not previously infected and not given drugs produced patent infections. Reinfection was accomplished in 2 of 3 trials. The prepatent periods

TABLE 2

Prepatency and patency of 26 experimental E. histolytica *infections in human volunteers observed one month or longer after inoculation of 2,000 to 4,000 cysts and terminated by treatment at the end of the study*

Subject	Days Prepatent	Months Patent	Stools Examined During Patency	
			Positive	Negative
P-1	4	1	18	0
P-2	7	14	42	7
P-3	5	9	32	5
P-7	14	14	27	21
P-8	5	9	29	4
P-9	3	6	33	4
P-11	9	14	35	5
P-12	9	3	23	4
P-13	126	10	18	0
P-14	5	14	44	8
E-7	*	11	18	5
F-1	*	11	21	0
F-2	*	8	18	2
F-3	*	11	17	0
F-4	*	11	16	7
F-5	*	12	20	2
F-6	*	1	5	0
F-7	*	12	18	0
G-1	5	12	17	4
G-2	12	4	9	2
G-3	5	3	9	0
G-4	5	12	17	4
G-5	5	3	10	0
G-6	5	7	12	2
G-7	5	3	10	0
G-8	5	12	19	2

P—Infected by preliminary trial of inoculation procedures.
E—150 mg, F—75 mg Milibis daily for 4 weeks, challenged 3 times.
G—Untreated controls.
* Prepatency influenced by chemoprophylaxis.

varied in the 48 infections from 2 days to 4 months. In all but 2 infections it was 14 days or less; in 33 (68.7%) it was 7 days or less. These findings are in general agreement with those reported earlier (Table 1).

2. *Stability of infections.* There was wide variation in the regularity with which amebae could be found in the stools during the patent periods (Tables 2 and 3). Among 33 individuals observed for a month or more, there were 10 from whom 9 to 24 stools were examined without failing to find amebae. In one whose infection was followed for 14 months (P-7 in Table 2) there were 3 periods of 4 to 7 weeks when the stools were consistently negative, then during the last 5 months they were consistently positive. In the majority, negative findings were few and widely scattered. As an over-all average, amebae were found in 86 per cent of

TABLE 3

Prepatency, patency and postpatency of 7 experimental E. histolytica *infections in human volunteers whose infections apparently disappeared without treatment*

Subject	Days Prepatent	Months Patent	Stool Examinations During			Months Observed Postpatent
			Patency		Postpatency	
			Positive	Negative	Negative	
P-4	14	2	14	1	33	12
P-5	26	1	7	5	37	13
P-6	4	4	24	0	20	10
P-10	11	2	11	0	32	12
E-2	5a	1	7	4	18	8
E-6	12b	8	12	3	6	4
F-8	6a	7	15	2	10	4

a—After second challenge during chemoprophylaxis.
b—After last challenge which was given 7 days before end of chemoprophylaxis.

the stools examined during the patent periods, i.e., between the first and last stools in which amebae were found.

Twenty-three infected individuals were observed for 9 to 14 months. In this group, 7 apparently lost their infections spontaneously. Two of these infections were patent for only 21 days (E-2) and 39 days (P-5) respectively; 2 persisted for 2 months, 1 for 4 months and the others for 7 and 8 months (table 3).

3. *Susceptibility to reinfection.* Three individuals who had spontaneously lost experimental *E. histolytica* infections after 39, 61, and 62 days respectively, and had been negative by stool examinations for 12 to 14 months (P-4, -5, -10 in Table 3), and 3 others not previously infected were inoculated with 4,000 cysts on 2 occasions a week apart. Prepatency was calculated as the period between the first challenge and the first positive stool. Due to unfavorable work schedules of the subjects, specimens could not be collected daily. The first to be examined from those not previously infected, 2, 6 and 9 days respectively after inoculation were positive. In one of the previously infected individuals (P-5) amebae were not found in 8 stools examined during the 4 weeks following inoculations while in the two others infection became evident 6 (P-10) and 10 (P-4) days after the first inoculation, and the stools were consistently positive for the following 4 weeks. The refractory individual's initial infection had a fairly long prepatency of 26 days and persisted for only 26 days (Table 3).

4. *Symptomatology and pathogenicity in human volunteers.* The first group of 14 volunteers (P-1 to -14) were given cysts washed from a stool of a carrier who for two years had had no detectable symptoms of amebiasis. For subsequent experiments cysts were derived from one of the first group (P-2) who similarly was a carrier without evident symptoms, then or later. Volunteers for infection were given physical examinations and questioned regarding complaints prior to inoculation. Physical examinations were repeated at 2- to 3-month intervals afterwards. The character of the stools and any complaints voluntarily reported

by the subjects were recorded routinely. Physical complaints were solicited at 2- to 4-week intervals. Similar examinations were carried out on 14 other uninfected individuals.

There was no dysentery in the experimental group and diarrhea was uncommon (8%). The incidence of diarrhea was somewhat higher in the uninfected group (14%). Mild abdominal tenderness, usually in the right upper quadrant and pain usually epigastric were found in 40 per cent of the infected volunteers both before and after inoculation. Men who were not infected presented these symptoms approximately as frequently (36 per cent in the initial examination; 50 per cent throughout the study). In no instance could symptoms be definitely related to the infections. One man (P-13) had consistently abnormally pale, soft and mucoid stools throughout a very long prepatent period but the condition may have existed prior to inoculation. Eleven individuals who complained of abdominal pain or right upper abdominal tenderness were treated although the symptoms were mild and probably due to other conditions. Blood in or on the stools was observed in 2 individuals. Sigmoidoscopy demonstrated in both cases bleeding hemorrhoids and abundant motile amebae but no ulceration of the colon.

In the course of the study 4,537 fecal specimens were examined and classified into 8 categories of consistency from hard (class 1) through mushy (class 4) to dysenteric (class 8). Of this number, including stools from non-infected individuals as well as those with amebic infections, either experimental or natural, 1.7 per cent were in class 1, 16.9 per cent in class 2, 64.7 per cent in class 3, 14.8 per cent in class 4, 1.2 per cent in class 5, and 0.6 per cent were in the distinctly diarrheic classes 6 and 7. Examination of prospective volunteers detected 15 with *E. histolytica* infections. One of these naturally infected individuals was observed to have acute amebic dysentery and one other gave a history of recurrent diarrhea; the remaining 13 were asymptomatic (Jung *et al.*, 1955).

In the experimental group diarrheic specimens were about equally frequent among infected and non-infected men and often were produced by saline purgatives which could be taken at will. Among the 33 infected individuals observed for 1 to 14 months, there were 9 (P-2, P-3, P-5, E-7, F-4, F-5, F-8, G-1 and G-5) who submitted 1 to 3 stools in classes 5 to 7 during the patent periods. Six of the 9 (P-2, P-5, E-7, F-4, F-5 and G-1) submitted 1 to 4 such stools before inoculation or after treatment. Excluding one special case (P-13), which submitted 17 abnormally soft stools during a long prepatent period but only formed stools during patency, 14 of 779 stools (1.8%) during infection and 9 of 526 (1.7%) before and after infection were diarrheic (including class 5).

In single doses, 4 volunteers were given respectively 10,000, 50,000, 500,000 and a million cysts from a previously infected volunteer (2 previous passages from original donor). The prepatent periods may have been shortened (48 hours in the two given 10,000 and 50,000) but otherwise the results were the same as from the inoculations of 2,000 or 4,000 cysts. All four subjects were symptomless during 6 weeks of observation.

5. *Pathogenicity in experimental animals.* Additional observations on the pathogenicity of the experimental strain ("H") of *E. histolytica* were made on dogs, guinea pigs and rats.

Dogs. Four dogs were put on a canned salmon diet 2 weeks before inoculation, and were continued on this diet throughout. Six others were fed horse meat for 10 days after inoculation, then switched to salmon. The dogs received 50,000 to 2 million cysts orally, washed from a fresh stool of the original donor.

One dog of the first series began passing trophozoites in bloody stools 6 days after receiving 1 million cysts. At autopsy on the ninth day small superficial ulcers containing amebae were evident in the mucosa of the upper colon. The second dog had bloody mucoid stools starting 11 days after inoculation. Amebae were not found in the stools and no evidence of infection was found at autopsy 4 days later. Hookworms in the small intestine and cecum apparently were responsible for the bloody stools. The other 2 dogs showed no evidence of infection although each was reinoculated with a million cysts and sacrificed after 11 days.

The second series of 6 dogs was inoculated with 50,000 to 125,000 cysts from one of the volunteers (F-2). Two of the dogs died 13 and 17 days after inoculation as a result of an undiagnosed (non-amebic) ulcerative condition of the mouth. Their stools were diarrheic or dysenteric but amebae could not be demonstrated in either the stools or the colon. Of the remaining 4, 2 passed only normal stools without demonstrable amebae for 6 weeks after inoculation and were discarded without autopsy examination. Another passed dysenteric stools occasionally for 2 weeks after he was put on a salmon diet but amebae could not be demonstrated and it was evident that the bloody stools were due to a maturing hookworm infection. The sixth dog in this series received approximately 125,000 cysts. Nine days after inoculation, 2 days before starting on the salmon diet, amebae were abundant in a diarrheic stool. Five days later the diarrhea graded into dysentery which rapidly became more severe up to the time of sacrifice 17 days after inoculation. Small bleeding ulcers containing amebae were numerous in the upper half of the colon and the cecum. However, blood and hookworms were plentiful in the small intestine where amebae were not found. Stained sections showed typical amebic lesions containing multitudes of invading amebae, although none of the amebae extended into the submucosa.

Guinea pigs and rats. These animals were inoculated with two strains of amebae, "H" and "P", from cultures. The "H" strain had been isolated from the carrier who provided cysts for chemoprophylaxis experiments and maintained by serial transfer for 15 months in LES medium. The "P" strain was isolated 2 months previous to the experiments, from a five-year-old girl with severe amebic dysentery of several weeks duration. Guinea pigs weighing 150 to 250 gm. and rats 40 to 45 gm. were used. The guinea pigs were maintained and inoculated as described by Carrera and Faust (1949); the rats were similarly inoculated but were maintained on a free-choice diet of whole-grain oats, bran and cabbage (Megraith and Harinasuta, 1954). Routinely 200,000 trophozoites from 48-hour cultures were inoculated intracecally. The guinea pigs were sacrificed 8 to 12 days and the rats 4 to 6 days after inoculation. Of the animals inoculated with the "H" strain, 18 guinea pigs and 10 rats survived the inoculation operation; of those inoculated with the "P" strain, 19 guinea pigs and 11 rats survived.

Five of the guinea pigs in the "H" series developed amebic lesions. On the scale

commonly used (Phillips and Bartgis, 1954), the ulceration was classified as ++++ in 1, ++ in 3 and + in 1. In the "P" series, 4 had amebic lesions, ++++ in 1 and + in 3. Amebae were not found in mucosal scrapings of animals without gross lesions. In rats, the "H" strain established infections in each of 10 trials but produced visible lesions in only 4, ++ in 1 and + in 3. The "P" strain produced infections in 8 of 10 rats and 6 had evident lesions: ++++ in 2, ++ in 1, and + in 3.

DISCUSSION

In previously uninfected, unmedicated individuals, 2,000 cysts or more invariably produced infections. Because of the importance of adequate challenges to chemoprophylactic trials the use of smaller inocula was not feasible. It is presumed that infections of *E. histolytica* may occasionally be established by the ingestion of one viable cyst as was demonstrated by Rendtorff (1954) in 1 of 8 trials with *E. coli*. However, he failed to establish infections in 7 of 10 volunteers given 10 cysts, in 2 of 4 given 100 cysts and in 2 given 1,000 cysts. Ten thousand cysts did not fail in either of 2 trials. In the present study the choice of 2,000 cysts for inoculation was based on judgment only, there being at that time no guiding data on this matter from previous studies on experimental infections in human volunteers.

Prepatency of amebic infections apparently is not markedly affected by the size of the inoculum. In *E. coli*, Rendtorff (1954) found the prepatent period to be 9 days after inoculation of 10,000 cysts and 5 to 8 days in 5 of 6 successful trials using 1 to 100 cysts. In the present study there was no sharp difference in results from inoculations ranging from 2,000 to 1 million cysts of *E. histolytica*.

One noteworthy instance of a very long prepatent period of 4 months was observed. It is not clear whether the inoculation resulted in a very small unthrifty colony that produced trophozoites and cysts in quantities below the efficiency threshold of the diagnostic technics employed, or the colony thrived but produced only trophozoites which invariably perished in the fecal stream. As already noted the stools of this individual (P-13) were abnormally pale and mucoid throughout the prepatent period and purged specimens were unsuccessfully examined on two occasions. Later, after the stools had changed to a more normal character amebae were consistently found. In addition to zinc sulfate flotation and the direct saline smear which were routinely employed, 7 stools at irregular intervals during the prepatent period were examined by the MIF (Sapero and Lawless, 1953), FE (Ritchie, 1948), PVA (Goldman and Brooke, 1953) technics and 17 specimens were examined by the iron-hematoxylin technic, without detecting amebae. A firm impression was gained that the stools were practically devoid of amebae in any stage in the stools until after, for no apparent reason, the stools became normal in consistency, color and elements. Nothing noteworthy was observed in the character of the feces of the individual whose infection was not diagnosed until 26 days after inoculation.

While in all experimental infections of *E. histolytica* in human volunteers the prepatent period has generally been less than two weeks, one of 18 infections

studied by Walker and Sellards (1913) did not become evident until 21 days, and 2 others were not found until 33 and 44 days although earlier examinations were too inadequate to establish the prepatent periods with confidence. Considering all of the 70 experimental infections in which prepatent periods could be established at least roughly, 96 per cent were diagnosed in 2 weeks or less and 71 per cent in 1 week or less after inoculation. The range in prepatency extends from 1 day to 126 days (Table 1).

It is of interest that routine stool examinations based on zinc sulfate flotation and the direct saline smear detected amebae in 86 per cent of the specimens examined after the infections became established. The efficiency of diagnostic technics have been calculated from the results of successive stool examinations or from two examinations made on fecal samples from the same individuals at different times (Tobie *et al.*, 1951; Mackie *et al.*, 1955). The success rate of 86 per cent as compared with approximately 60 per cent previously reported may be an indication that the strain of amebae employed in the present study was above average with respect to its production of cysts and diagnosable trophozoites. More likely, the difference is a reflection of the uniformity and quality of the diet provided the experimental subjects as compared with the average individual, along with the regularity of habits imposed on them by the prison routine. Stools from this group were remarkably uniform and normal.

The donor of the cysts used for experimental inoculations and for establishing the strain of *E. histolytica* in cultures was seen 34 times between February 1952 and July 1955. His presenting complaint was pain over the heart upon exertion; noteworthy intestinal disorders including diarrhea were denied. He was given laboratory and physical examinations on 4 occasions, including sigmoidoscopy twice. One one occasion, February 1952, fine pinpoint hemorrhages in the upper rectum and lower colon were seen and amebae were present in material aspirated from the colon. In May 1952, slight tenderness over the cecum was noted and considered of possible significance. In 41 months, 36 normal stools and one series of purged specimens were examined. *E. histolytica* cysts were abundant or numerous in 12 specimens, moderate to scanty in 20 and were not found in 4. In the last 4 specimens examined (July, 1955) cysts were recorded as scanty. Clinical indications of amebiasis have been lacking, with the possible exceptions mentioned.

The general high level of health, food and water sanitation, and the rigid pattern of living possibly contributed to the apparent lack of pathogenicity of the "H" strain in human volunteers. In dogs, guinea pigs and rats it was capable of tissue penetration and was as pathogenic as a recently isolated strain deemed at that time to be highly pathogenic in a five-year-old child. Neither strain exhibited a high degree of pathogenicity although they compared favorably with strain "200" used in recent studies by Rees *et al.* (1954) and Krupp (1956).

E. histolytica infections in human volunteers studied by Simic (1931) did not produce symptoms while under observation, but they were followed for only 2 months, or less. Westphal's (1937) one case was followed for 8 months after inoculation of cysts from a man who was previously symptomatic. During this

time there were two periods of intestinal disturbance accompanying a cold, and occasional slight diarrhea occurred during the warm summer months. These disturbances were regarded as unimportant. When near the end of the 8 months period a bacterial infection (Flexner Y) was superimposed on the amebic infection, frank dysentery developed but subsided without anti-amebic therapy. Of the 18 experimental infections in prisoner volunteers studied by Walker and Sellards (1913), 4 became symptomatic with the onset of dysentery occurring 12, 57, 87 and 95 days after inoculation. In two cases, the symptoms were mild and transient, requiring no treatment. One of the treated cases relapsed, but thereafter was asymptomatic for 9 months or longer while cysts persisted in the stools. In the other symptomatic case, in which the onset of symptoms was 57 days after inoculation, the dysentery persisted 2 weeks before treatment was given and did not relapse during 2 months of observation. This study was made on men who, like those of the present study, were prisoners (Bilibid Prison, Manila, P. I.). They ate cooked food and drank distilled water exclusively and were under more or less constant observation. Unlike the present group, they were inoculated with 4 different strains of amebae, 3 that had at one time produced acute amebic dysentery. However, all of the inoculated cysts came from individuals who had been asymptomatic for 2 months or longer and one of the 4 symptomatic cases was produced by the "carrier" strain. The pathogenicity or virulence of different strains of *E. histolytica* is one of the problems of greatest current interest (Anderson *et al.*, 1953; Faust, 1954; Rees, 1955). It has been maintained that some strains, and perhaps all strains under certain circumstances, are essentially non-pathogenic (Hoare, 1952). Experiences thus far with *E. histolytica* in human volunteers support that view. With reference to the "H" strain used in the present study, although it failed to produce recognizable disease in either the original donor or a considerable number of human volunteers, its behavior in experimental animals does not permit regarding it as non-pathogenic.

SUMMARY

Eighty-one experimental *Entamoeba histolytica* infections were studied in human volunteers at a state penitentiary. Inoculation of 2,000 to 4,000 cysts derived directly from a non-symptomatic "carrier" or after one or two passages through volunteers, produced infections in 42 of 42 individuals not medicated or previously infected; heavier inocula did so in 4 of 4 others.

Prepatent periods, based on zinc sulfate flotation and saline smear fecal examinations, varied from 2 days to 4 months. In all but 2 of 48 individuals the stools were positive within 2 weeks; 68.7 per cent were positive in 7 days or less. An extraordinarily long prepatency of 4 months in one case was associated with abnormally pale, mucoid stools.

From 33 individuals followed for more than 1 month after infection, 86 per cent of the stools examined were positive. In one there were 3 periods of 4 to 7 weeks when amebae could not be demonstrated. Among 23 infected individuals observed for 9 to 14 months there were 7 who apparently lost their infections

spontaneously after 3 weeks to 8 months. One of 3 individuals who had spontaneously lost infections was refractory to reinfection when given two inoculations of 4,000 cysts.

None of the volunteers developed symptoms that could be associated with the amebic infections. However, the strain of amebae used in the human experiments could not be regarded as non-pathogenic, since it produced typical amebic lesions in dogs, guinea pigs and rats, and was as pathogenic in guinea pigs and rats as a recently isolated strain from a frankly dysenteric patient.

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INTESTINAL PARASITES IN AN EGYPTIAN VILLAGE OF THE NILE VALLEY WITH EMPHASIS ON THE PROTOZOA[1]

DEANER K. LAWLESS, ROBERT E. KUNTZ AND C. P. A. STROME

Parasitology Department, Naval Medical School, NNMC Bethesda, Maryland

As a preliminary step in the study of disease among the peoples of Egypt, the present survey was undertaken to determine the incidence of intestinal parasites, especially the protozoa, in a typical village in the irrigated part of the Nile Valley. El Kunaiyisa, a small village 8 to 10 miles southwest of Cairo, in Giza Province and within view of the pyramids, was chosen because of accessibility and the fact that other work by NAMRU-3 was in progress in this area. Furthermore this village represented the typical environment in which parasite propagation is highly efficient.

When initiated, this project was set up to make a linear study of the incidence and fluctuation of parasite population in the same group of individuals for several years. For this purpose 100 persons, willing to submit stool specimens for a series of surveys, were chosen at random, the majority falling in the age of 5 to 30 years. This sample, involving the members of 13 families, probably constituted 4 to 7 per cent of the village population. As might be expected in a rural population of this type, it was impossible to contact the entire group on each survey since some had moved away and several had died. Although the number varied from one survey to the next, 80 of the original lot submitted fecal specimens for each of six surveys, forming the basis of this report.

MATERIALS AND METHODS

One fresh preparation was made from each stool specimen which had been deposited in a labeled sputum cup. Although the stools were taken directly to the laboratory, frequently 2 to 3 hours elapsed between time of collection in the village and examination in the laboratory. A direct smear using saline-iron hematoxylin solution (Lawless, 1946) was employed for detection and preliminary recognition of protozoa. This method was especially used to diagnose trophozoites of the flagellates and *Dientamoeba fragilis*. Permanent smears prepared by the iron hematoxylin stain technic were employed for a more thorough search for all parasites and especially for confirmation of species identification. The latter method was imperative for accurate identification of *Entamoeba*

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the view of the Department of the Navy or the naval service at large.

This is one of a series of reports on surveys of intestinal parasites of man in Egypt and the Middle East, made while the authors were serving as members of the staff of the Department of Parasitology, U. S. Naval Medical Research Unit, No. 3 Cairo, Egypt.

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histolytica. Although cysts and trophozoites of the same species were often present, identification of all protozoan infections was based on cysts, except in the case of *D. fragilis* and *Trichomonas hominis*. Although the time of examination varied, approximately 20 to 25 minutes were spent on the study of each preparation. In this survey the 'large' and 'small" races of *E. histolytica* were recognized; the latter designation applied to cysts consistently measuring less than 10μ.

Although it was realized that standard brine flotation has definite limitations, this technic was employed for concentration of helminth eggs with a third sample from each stool. When feasible a total of 6 specimens was obtained at different times from each individual. Collections were made at different intervals over a period of 30 months (1948–1950).

DISCUSSION

Although this survey is of a somewhat limited scope in the sense that it was not carried to completion as originally planned, it reveals an unusual incidence of parasites in man. Table 1 shows the incidence of parasites detected by a careful study of stool specimens obtained from a small sample of the agricultural population of Egypt during the course of 6 surveys over a period of 2½ years. Since the group under consideration was small, no attempt was made to segregate participants according to age, sex, occupation, familial relationship, location of home within village or other categories which in larger population samples would undoubtedly reveal additional epidemiological information.

The range of incidence for the different parasites from one survey to the next is considerable, but perhaps not surprising in view of the long intervals between surveys and the fact that studies were made during different seasons of the year. Figures for the incidence of infection based upon 6 surveys are highly significant and surpass considerably rates usually quoted for incidence of parasites in Egypt or elsewhere. As intimated by Abdel Azim (1932) and more recently by Halawani *et al.* (1951), such high rates of infection are closely related to the state of sanitation of the community under study.

An incidence of more than 90 per cent for *Entamoeba histolytica*, *E. coli* and *Endolimax nana*, although based on 6 surveys, seems preposterously high but it is acceptable when one looks to the works of other investigators in this field for support. Halawani *et al.* (1951) found *E. histolytica* in 30 to 85 per cent of 400 specimens in a survey made in the same general area near Cairo, and Chandler (1954) detected this parasite in 55 and 59 per cent of the population of two villages in Qalyubiya Province a few miles north of Cairo. In the latter study, based upon a single sample from each person, he estimated that he probably found no more than 60 per cent of parasites actually present. We concur in Chandler's statement that "practically, if not actually, 100 per cent of the older children and adults harbor *E. histolytica*". The present survey supports the contention of Svensson (1935) and others that at least 6 examinations are necessary to obtain a fairly accurate incidence of parasites in a given population. In general our figures of incidence for most of the intestinal protozoa are

TABLE 1

Incidence (per cent)[1] *of intestinal parasites based upon examination of 6 fecal samples obtained at intervals from the same 80 persons in an Egyptian village*

Parasites	Incidence at Each Survey						Total Positives Revealed by the 6 Surveys
	Jan '48	Sep '49	Nov '49	Apr '50	May '50	Jun '50	
Protozoa							
Entamoeba histolytica (LR only)[2]	11	16	9	18	7	17	3[3]
Entamoeba histolytica (SR only)	19	23	34	20	22	23	14[3]
E. histolytica (LR & SR together)	36	20	22	15	23	21	80
Total *E. histolytica*	66	59	65	53	52	61	97
Entamoeba coli	86	66	70	63	69	72	98
Endolimax nana	60	44	49	52	50	44	93
Iodamoeba bütschlii	24	22	15	26	19	24	65
Dientamoeba fragilis	9	1	2	1	0	1	11
Giardia lamblia	18	5	9	10	15	20	40
Chilomastix mesnili	25	15	11	7	14	13	55
Trichomonas hominis	4	4	9	13	13	9	35
Enteromonas hominis	22	33	32	15	20	23	74
Helminths							
Hookworm (*Ancylostoma*)	—	52	33	34	35	36	71
Ascaris lumbricoides	—	25	16	30	39	31	51
Trichuris trichiura	—	2	2	3	3	2	9
Enterobius vermicularis	—	14	8	18	19	17	59
Hymenolepis nana	—	9	10	11	7	6	23
Trichostrongylus sp	—	22	16	33	34	28	70
Strongyloides stercoralis	—	1	0	1	0	0	1

[1] Given as nearest whole number.

[2] LR—Large race *E. histolytica* with cysts consistently measuring more than 10 μ; SR—cysts consistently less than 10 μ.

[3] Percentage of people showing only one and the same race of *E. histolytica* on all 6 examinations.

comparable to those listed by Chandler (1954) and Wells and Blagg (1956) also working in Egypt. Note should be made of the unusually high incidence for *Enteromonas hominis*.

Although there were complaints of diarrhea and other intestinal ailments, it was impractical in the present survey to correlate the presence of 'large' and 'small' race *E. histolytica* with clinical manifestations. However since there has been a trend toward the recognition of races of *E. histolytica* based on pathologic, morphologic and serologic grounds, we have made our determinations accordingly. In this respect it is of importance to note that only 3 per cent of persons,

based upon 6 surveys, were infected with the large race *E. histolytica* alone and 14 per cent with the small race. Halawani and coworkers (1951) have also recognized the presence of two races of *E. histolytica* in parasites recovered from their survey. From this group they reported that 72 per cent were of the small race and 28 per cent of the large race, cysts of the latter measuring 11 to 15μ. These proportions are similar to those given in Table 1.

Incidence of helminth parasites is also remarkably high, even in view of the parasite-prosperous environment from which study specimens were obtained. *Ascaris* and hookworm have been recognized as two of the most common nematode parasites of man in Egypt for years, and the incidence reported here for *Ascaris* is comparable to that given by Chandler (1954), although our figures for hookworm are somewhat greater. *Trichuris* is not as common as might be expected, but the eggs of this species probably are more prone to destruction than those of *Ascaris* or *Ancylostoma*. We are unable to account for the incidence of 59 per cent for *Enterobius vermicularis* obtained by stool examination only, unless this village just by chance, is more heavily seeded than some of the others in which survey work has been conducted (Kuntz, Lawless, unpublished data). *Trichostrongylus* infection is high (70 per cent) but none too surprising since the people in villages of this type are closely associated with numerous domestic animals housed under the same roof. A single infection with *Strongyloides* was detected.

It is unnecessary to go into details of relationship of environmental conditions, standards of sanitation, nutritional deficiencies, etc. to parasite infection since these aspects have been adequately described by Weir *et al.* (1952), Chandler (1954) and various Egyptian investigators.

SUMMARY

The incidence of intestinal parasites, with emphasis on the protozoa, in a small sample of the agricultural population of the Nile valley in Egypt was determined by study of stool specimens. A single specimen was taken on 6 separate surveys over a period of 2½ years. Figures for incidence of the protozoa and most of the helminths are unusually high. *Entamoeba histolytica*, *E. coli* and *Endolimax nana* occurred in 97, 98 and 93 per cent of specimens respectively. *Enteromonas hominis*, a protozoan frequently overlooked, was detected in 74 per cent. Fourteen per cent of the people had the small race of *E. histolytica* alone, whereas only 3 per cent were infected with the large race alone. Hookworm and *Trichostrongylus* eggs were found in 71 and 70 per cent respectively of specimens examined, *Ascaris* in 51 per cent and *Enterobius vermicularis* in 59 per cent. Such a high incidence of infection reflects the living conditions of the people from whom the material for examination was obtained.

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EXPERIMENTAL CHEMOPROPHYLAXIS OF AMEBIASIS[1]

PAUL C. BEAVER, RODNEY C. JUNG, HARRY J. SHERMAN, THOMAS R. READ AND THOMAS A. ROBINSON[2]

Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, Louisiana, and Medical Department, Mississippi State Penitentiary, Parchman, Mississippi

The frequency of *Entamoeba histolytica* infection contracted by travelers in endemic regions outside the United States prompted Craig (1940) to observe that while attention to hygienic rules undoubtedly would prevent most of these infections, it is often impossible to follow hygienic rules, and if an efficient drug could be employed as a prophylactic, its use should prove to be a marked advance in the prevention of amebiasis. He suggested Diodoquin (5,7-diiodo-8-hydroxyquinoline) as the best drug for that purpose since it is absorbed slowly from the intestinal tract and its use is not accompanied by disagreeable symptoms even when administered in large doses over long periods of time. He recommended the same daily dosage as for the treatment of cyst passers (1.47 gm.) but later (1944) stated that 0.42 gm. taken twice daily during, and for one week following, the period of exposure probably would be sufficient to destroy any amebae that might be ingested, and that the treatment could be continued for a month or more if necessary. Craig's suggested regimen was not tested experimentally.

At the end of World War II the incidence of amebiasis was significantly lower in United States troops returning from the Pacific area than from Europe and North Africa (Jacobs *et al.*, 1946). Radke (1951, 1953a) noted that one of the differences between the two areas was the widespread employment of Atabrine (quinacrine hydrochloride) as a malarial suppressive agent in the Pacific, and he inferred that in addition to suppressing malaria, Atabrine afforded significant protection against *E. histolytica* infections. Certain experimental and clinical investigations of Atabrine as an amebicidal agent were interpreted as lending support to the above inference (Radke, 1951, 1953b; Bernstein, 1946).

Milibis (bismuth-glycolylarsanilate) frequently is ineffective in the treatment of frank amebic dysentery (Wilmot *et al.*, 1951), but it rarely fails to eradicate amebic infections from non-dysenteric patients when given in the recommended dosage (Berberian *et al.*, 1950; Sodeman and Beaver, 1952). The high cure rates obtained with Milibis, together with the observation that, like Diodoquin, it is relatively insoluble in the bowel and of low toxicity in curative dosage, early suggested its suitability for prophylactic use. At first, its prophylactic application was conceived as periodic administration of curative dosages to known or

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[2] Address, Medical Dept., State Penitentiary, Parchman, Miss.

possible cyst passers, thereby reducing the rate of new infection and removing the danger of frank disease which might develop from the carrier state (Hauer, 1943; Westphal, 1948; Wagner, 1951). For closed populations such as institutional groups, a prophylactic program consisting of initial treatment of all residents, followed by routine treatment of all newly admitted or readmitted individuals has been used with apparent success in one instance (Sodeman and Beaver, 1952). In less than the recommended curative amount, Milibis (in combination with chloroquine phosphate) has twice been tested as a prophylactic against amebiasis. Berberian *et al.*, (1952) gave it to patients in a mental hospital where the rate of natural transmission was high, and found that 1.5 gm. given in 3 divided doses every 7 days, or 0.5 gm. given daily for 10 to 14 weeks, markedly reduced the rate of reinfection. Encouraging results were reported by Hoekenga (1952) who tested it on an open population in Honduras, giving as an adult dose 750 mg. daily on 2 consecutive days each week for 12 weeks. In neither of these studies was it possible to determine the frequency or intensity of exposure.

In the present study the objective was to determine the minimum daily doses of Milibis and Diodoquin that would give protection against *Entamoeba histolytica* infection in individuals who were ingesting at predetermined intervals known quantities of viable cysts. In addition, the prophylactic effects of Atabrine and chloroquine diphosphate in the dosages recommended for the suppression of malaria were tested against experimental inoculations of *E. histolytica*.

MATERIALS AND METHODS

E. histolytica cysts were obtained from a 16-year-old white male who for two years had been a consistent cyst passer, without symptoms of amebiasis. The cysts were morphologically typical of large race *E. histolytica* and established vigorous colonies in standard culture media. Infection experiments were carried out in the Mississippi State Penitentiary where male prisoners were permitted to participate as volunteers. No inducements were offered, and the only rewards were cigarettes on special occasions and carbonated beverages which the subjects knew were sometimes contaminated with infective organisms. The prisoners were not isolated and were not excused from their regular routine. On rare occasions, due to ineptness (illiteracy) there were troublesome inaccuracies in the identification of specimens. Four of the subjects had to be withdrawn because of purposeful deception and one was lost by suspension of sentence.

The Mississippi State Penitentiary at Parchman is primarily an agricultural institution divided into 18 individual work-camps of approximately 125 men each. The men sleep in barrack-like dormitories, each accommodating approximately 60 men. The quarters are somewhat crowded, causing considerable close contact among the men. Each camp has a separate dining room, and the food handlers in the kitchens and dining rooms are prison inmates. Adequate toilet facilities and showers are provided at the dormitories, but no sanitary provision is made for men at work in the fields.

Examinations of fresh stools were made in a laboratory established for that purpose at the prison. Fecal specimens were collected in labeled cardboard boxes

under the direction of camp supervisors. Most of the collections were made from morning stools and were transported to the laboratory within two hours. All specimens were concentrated by zinc sulfate centrifugal flotation and examined in iodine stain as previously described (Beaver, 1952). Supplementary technics such as formalin-ether sedimentation (Ritchie, 1948) and direct saline smears were used when indicated by the condition of the stools. For independent confirmatory study, fecal smears frequently were fixed in Schaudinn's solution and stained with iron-hematoxylin.

A preliminary survey based on single stool examinations revealed *E. histolytica* in 2 to 4 per cent of the men in the various camps. Because of this relatively low infection rate it was assumed that naturally acquired infections would have no important influence on most phases of the study. However, volunteers were rejected if *E. histolytica* infections were found. When accepted, each was given a physical examination, and his clinical history was recorded.

In the first series of experiments (28-day trials) cysts for inoculation were concentrated from fresh feces and washed by repeated centrifugal sedimentation in water. For all remaining trials the inocula were washed as above, suspended for 30 minutes in a 1-part-per-million solution of chlorine, then washed again to remove the chlorine.

The drugs were administered under supervision in the following milligram doses: Milibis, 500, 250, 150 and 75 once a day for 28 days and 250 once a day for 21 days; Diodoquin, 650, 325, and 210 once a day for 21 days; Atabrine, 500 per day for 2 days, then 100 once a day for 21 days; and chloroquine 500 per day for 3 days, then 500 once a week for 3 weeks. Controls were given placebos of 330 mg. sodium bicarbonate.

The experiments usually were set up with 8 test individuals for each dosage trial and 8 controls for each batch of inoculated cysts. Three or more stool examinations were made on each individual immediately prior to the trials. During the period of drug administration, challenges of 2,000 cysts were given at the end of the first, second and third weeks of the 28 day trials, and 4,000 cysts were given at the end of the first and second weeks of the 21 day trials; i.e., the drug was given one week before the first weekly challenge and was continued one week beyond the last. Two to three stools were examined weekly during the test period and for the next 4 to 7 weeks, then 3 daily stools were examined every 3 to 4 weeks, except in the last Diodoquin series for which the follow-up period was only 4 weeks beyond the last challenge.

At the termination of the study, regardless of findings on stool examinations, all volunteers were treated with a combination of Milibis (1.5 gm. per day for 14 days) and chloroquine (100 mg. daily for 2 days, 50 mg daily for 14 days), and followed by 3 stool examinations every 4 weeks for 3 months.

RESULTS

In the 28-day trials of Milibis, 48 individuals were divided into 6 groups of 8 each. Four groups were given respectively 500, 250, 150 and 75 mg. of Milibis daily and 2 were given placebos. All individuals of the control groups became infected, mostly within one week after exposure. In none of the individuals of the

500 and 250 mg. groups were amebae ever demonstrable. Infections were demonstrated in 3 individuals of the 150 mg. group, and in all of the 75 mg. group (Table 1). The 3 infected individuals in the 150 mg. group remained negative while on the drug but were positive when examined on the sixth post-treatment day. In the 75 mg. group, 5 became positive during the test period—2 after the first, 2 after the second and 1 after the third challenge. The other 3 became positive within one week after the drug was discontinued (Table 2).

An additional trial of the 250 mg. dose, for 21 days, was started on 16 test individuals and 8 controls. Two of the men in the test group became uncooperative and were dropped from the experiment. Thirteen of the remaining 14 individuals were protected, while all of the controls were passing cysts 4 to 11 days

TABLE 1

The prophylactic effect of several drugs in various dosages tested on human volunteers inoculated with 2,000 to 4,000 Entamoeba histolytica *cysts*

Drug	Single Mg. Dose	Medication	Weekly Challenges	Volunteers Tested	Prophylactic Failures
Milibis	500	daily, 28 days	3	8	0
	250	daily, 28 days	3	8	0
	250	daily, 21 days	2	14	1*
	150	daily, 28 days	3	8	3
	75	daily, 28 days	3	8	8
Diodoquin	650	daily, 21 days	2	9	0
	325	daily, 21 days	2	7	3
	210	daily, 21 days	2	3	2
Atabrine	100	daily, 21 days†	2	8	8
Chloroquine	500	weekly, 4 weeks†	2	8	8
Placebo	333	correspondingly	1–2	35	34‡

* The unprotected individual's infection disappeared during a course of 250 mg. Milibis daily for 11 days and did not reappear during 3 months of observation.

† After higher daily loading doses.

‡ The uninfected control earlier had spontaneously lost an experimental infection (see Beaver *et al.*, 1956).

TABLE 2

The influence of prophylactic medication on the prepatent periods of experimental E. histolytica *infections in human volunteers*

Drug	Daily Dose in Mg.	No. of Individuals		Individuals Becoming Positive in				
		Inoculated	Infected	Test Period		Weeks after Discontinuance of Drug		
				Early	Late	1st	2nd	3rd
Controls	—	35	34	26	8			
Chloroquine	500	8	8	5	3			
Atabrine	100	8	8	3	5			
Milibis	75	8	8	2	3	3		
	150	8	3	—	—	3		
	250	22	1	—	—	1		
Diodoquin	210	3	2	—	—	2		
	325	7	3	—	—	—	1	2

after the first challenge. Cysts were found in the feces of the infected member of the test group 12 days after the last challenge, 5 days after the drug was discontinued. This individual possibly failed to swallow a portion of the medication, however, since 250 mg. of Milibis given under special supervision for 11 days terminated the infection. Eight other individuals, from the control group, who had become positive soon after challenging and had passed cysts regularly for one month, were given 250 mg. daily for 21 days. All became negative during the first week of therapy, and a follow-up period of three months failed to discover any relapses among them.

As a preliminary trial of Diodoquin, 3 individuals were given 210 mg. and 3 others 650 mg. daily for three weeks with challenges at the end of the first and second weeks. On the lesser dosage 2 were positive on the third day after the drug was discontinued; in the other, amebae were not found during a 3 months follow-up period. The 3 individuals given the 650 mg. dosage were negative for amebae throughout the 3 months period of follow-up observations. Following the preliminary observations, trials of 325 mg. and 650 mg. daily doses for 3 weeks were started with 8 men in each of two test groups and one group of 6 controls, including 3 individuals who had spontaneously lost their *E. histolytica* infections several months earlier. Of those given 325 mg., one was discharged from the prison and 2 in the 650 mg. group were lost because of non-cooperation. Three of the remaining 7 on the lesser dosage became infected, while 4 remained negative during the 4 weeks period of observation following the last challenge. The 6 individuals on the 650 mg. dosage remained negative for amebae while all except 1 (who previously had lost an infection) in the untreated group were positive within 10 days after the first challenge. In summary, 1 out of 3 was protected by the daily administration of 210 mg., 4 out of 7 by 325 mg., and all of 9 by 650 mg. of Diodoquin. (Tables 1, 2).

Atabrine and chloroquine each were tested on groups of 8 individuals. In the Atabrine-treated group, 3 had patent infections a week after the first challenge and the 5 others within a week after the second. In the chloroquine-treated group, 5 were positive in 1 week, 3 within 2 weeks after the first challenge. Seven of the 8 controls were positive within one week, while the remaining one was positive 4 days after the second challenge.

DISCUSSION AND CONCLUSIONS

The adult dose of Milibis currently recommended for the treatment of amebiasis is 500 mg. three times daily for 7 or 8 days. It would appear that the effective prophylactic dose is one-sixth that amount. On the particular strain of amebae used for the present prophylactic trials, 500 mg. given daily and continued for 1 week after the last of a series of relatively heavy inoculations of *E. histolytica* cysts barred infection in all of 8 test individuals. Daily doses of 250 mg. barred infection in 21 of 22 volunteers. The one failure is questionable inasmuch as daily doses of 250 mg. of the same drug terminated the infection in 11 days or less. The same dosage (250 mg. for 21 days or less) was curative when given to 8 other individuals who previously had been experimentally infected. Partial protection was provided by 150 mg. daily. Probably 250 mg. daily is an adequate

dose for ordinary prophylactic use. Under some circumstances a higher dosage may be required. Berberian *et al.* (1952) had 6 apparent failures among 63 individuals while on a regimen of 500 mg. daily in an institution where exposure was presumably more or less constant and amebae from diverse sources probably were involved. The six failures may possibly be due to the ineffectiveness of Milibis in the presence of diarrhea. However, the condition of the stools was not reported, and no other explanation for the failures is at present apparent. Reinfection following the period of medication must be ruled out by the nature of the procedures followed in the trials.

No support can be given to Radke's view (1951, 1953a) that Atabrine contributed to the relatively low incidence of amebiasis among military personnel in areas where it was used as a malaria-suppressive agent. The dosage of neither Atabrine nor chloroquine employed for that purpose had any detectable influence on experimental amebic infections in the intestinal tract when inoculations were given less than two weeks after prophylactic medication was started. Longer periods possibly would have altered the results.

The prophylactic dose of 840 mg. per day of Diodoquin which Craig (1944) suggested on an empiric basis, has been substantiated experimentally. Although the test group included only 9 individuals, and the period of observation following the last inoculation of cysts was relatively short, the tentative conclusion can be drawn that a somewhat smaller dose of 650 mg. per day probably would offer adequate protection under usual circumstances. Since even lesser amounts of Diodoquin as well as relatively small doses of Milibis (150 mg.) had a definite suppressive effect on amebic infections, such low doses during the period of exposure, followed by a week of heavier dosage possibly would be an effective and economical regimen.

SUMMARY

Two amebicidal drugs, Milibis (bismuth glycolylarsanilate) and Diodoquin (5,7-diiodo-8-hydroxyquinoline) were administered in less than the recommended curative doses to human volunteers who, during the period of medication, were periodically challenged with 2,000 to 4,000 viable cysts of *Entamoeba histolytica* derived from a non-symptomatic carrier. Starting one week before the first of three weekly challenges and continuing for one week beyond the last, daily doses of Milibis ranging from 75 to 500 mg. were tested in groups of 8 individuals, with a like number of controls for each series of tests. All controls became infected within two weeks after the first inoculation. The highest dosage gave complete protection, the least failed entirely, and 5 of the 8 test subjects were protected by a dosage of 150 mg. All of the 8 subjects given 250 mg. daily for 28 days remained negative. Additional trials of 250 mg., using a 21-day course with inoculations of 4,000 cysts given at the end of the first and second weeks of medication, resulted in one apparent failure among 14 test subjects. However, when the same dosage (250 mg.) was given to the infected individual and to 8 infected controls, all of the infections were terminated.

Similar experiments with Diodoquin resulted in complete protection of 9 individuals on 650 mg., 4 out of 7 on 325 mg., and 1 out of 3 on 210 mg. daily.

In all of 8 individuals given Atabrine (quinacrine hydrochloride) in doses of 500 mg. daily for 2 days followed by 100 mg. daily for 21 days, inoculations resulted in infections as readily as in the controls. Chloroquine given in weekly doses of 500 mg., after loading doses of 500 mg. daily for 3 days, likewise had no apparent prophylactic effect on experimental amebiasis.

Under usual conditions of exposure, effective prophylaxis of amebiasis probably can be accomplished by daily administration of 250 mg. of Milibis, or 650 mg. of Diodoquin, one-sixth to one-third the recommended curative doses. In the dosage recommended for suppression of malaria, Atabrine and chloroquine apparently have no prophylactic value against amebiasis.

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EXPERIMENTAL TRANSMISSION OF YELLOW FEVER BY CENTRAL AMERICAN SPECIES OF *HAEMAGOGUS* AND *SABETHES CHLOROPTERUS*

PEDRO GALINDO, ENID DE RODANICHE AND HAROLD TRAPIDO[1]

Gorgas Memorial Laboratory, Panama, R. P.

The invasion of Middle America by sylvan yellow fever in the years since its appearance in central Panama in 1948 has raised the problem of natural vectors in this region other than those known to transmit the disease in South America. The experiments reported here were intended to throw light on this problem. Studies on the forest mosquito fauna have shown that the known South American vectors with ranges which extend into Middle America (*Haemagogus spegazzinii falco* and *Aedes leucocelaenus* ssp.) have been either uncommon, rare or absent in certain of the places where yellow fever has recently occurred (Trapido and Galindo, 1955, and Galindo and Trapido, 1957a). The South American *Haemagogus* mentioned above is replaced in Middle America by other species. Most widespread is *Haemagogus equinus* which occurs from Venezuela and Colombia to southernmost Texas (Galindo and Trapido, in preparation). It is present on both the Atlantic and Pacific slopes. Other forest-inhabiting *Haemagogus* of Middle America are two of the three subspecies of *Haemagogus mesodentatus*, the typical race *mesodentatus* occurring on the Atlantic slope from western Panama to southeastern San Luis Potosi, Mexico, and the race *gorgasi* on the Pacific side from El Salvador to southern Sinaloa, Mexico (Galindo and Trapido, 1956 c).

In explanation of certain epidemiological aspects of the disease in Panama we have been much interested in determining the possible role of the sabethine mosquito, *Sabethes chloropterus*. Early in the work it was realized (Galindo, Trapido and Carpenter, 1950) that if this mosquito were shown to be a vector there would be a possible explanation for survival of the virus over the dry season. This matter of the mechanism of the survival of virus over the dry season has been elaborated in recent papers (Galindo, Trapido, Carpenter and Blanton, 1956 and Trapido and Galindo, 1957).

Of the species tested in this study *Haemagogus spegazzinii falco* and *H. equinus* previously had been the subjects of experiment. Both the typical subspecies of *spegazzinii* and the race *falco* had repeatedly been shown to transmit in the laboratory, and had been implicated in transmission in nature. The work with this species has recently been reviewed by Whitman (1951). In the case of *H. equinus*, material of Colombian origin was used by Waddell and Taylor (1945 and 1947) in various experiments in which successful transmissions were obtained. In the field studies made in South America virus was never recovered with certainty from naturally infected individuals. In Colombia Boshell-Manrique and Osorno-Mesa (1944) demonstrated the presence of virus in a group of three species of *Haemagogus*, one of which was *H. equinus*, but it is uncertain which of

[1] Present address: The Rockefeller Foundation, 49 West 49th Street, New York, N. Y.

these species may have been infected. The role of the latter species in nature has therefore been in doubt.

Interest in the sabethine mosquitoes stems from the recovery of yellow fever virus from a pool of four genera of this tribe by Shannon, Whitman and Franca (1938). One mosquito of the tribe, *Trichoprosopon frontosus*, is mentioned as having experimentally transmitted by bite (Waddell, 1949 and Whitman, 1951) but the details of the experiments have not been published. The difficulty in keeping these mosquitoes alive in the laboratory and inducing them to bite has limited the work with them.

MATERIALS

Virus strains. It seemed desirable to obtain a recently isolated strain of yellow fever virus which had undergone as few passages in experimental animals as possible. As the Trinidad Regional Virus Laboratory had made a number of isolations during the recent outbreak there, such a strain was solicited from the director, Dr. Wilbur Downs. He kindly forwarded to us the two strains used in the experiments to be described.

The first strain was received in dry ice on December 4, 1954 as mouse brain suspensions representing the first and second mouse passages of specimen ♯4754, the serum of a nonfatal case of yellow fever. This strain failed to produce fever or other symptoms in any of three rhesus monkeys injected by the subcutaneous route and circulating virus titers were low, although the monkeys later showed a high level of immunity. This strain was used only in the first experiment.

The second strain of yellow fever virus was received on March 14, 1955 as a suspension of the spleen of the original *Alouatta* (howling monkey), specimen ♯4204, found dead in the forest on July 30, 1954. This strain has proven uniformly fatal to rhesus monkeys and gives high circulating titers. It was used in the second and third experiments.

Experimental animals. White Swiss mice, usually 21 to 30 days old, were used for virus titrations and for isolation of virus from mosquitoes by injection. The intracerebral route of inoculation was employed.

Monkeys were used as sources of infective blood for the initial engorgement of the mosquitoes and as hosts for experimental transmission by bite. In the majority of the experiments juvenile rhesus monkeys were used. In a few experiments locally captured red or black spider monkeys (*Ateles geoffroyi robustus* and *panamensis*) were employed. All monkeys were previously tested for neutralizing antibodies by the intracerebral mouse protection technique.

Mosquitoes. As *Aedes aegypti* has presumably been eradicated from Panama, a colony of this species is not maintained at the Gorgas Memorial Laboratory. In view of the known efficacy of *Haemagogus spegazzinii* and *equinus* in transmitting yellow fever virus, these species were considered as controls.

The *Haemagogus spegazzinii falco* used in the experiments were the first generation progeny of females collected at Cerro La Victoria, near Panama City. This species was used only in the first experiment since it could not be colonized and since the numbers of females which could be collected was not large.

In the first experiment, the *Haemagogus equinus* used were both the progeny

of females collected at Cerro La Victoria, and stock from a colony which had been established with material from the same place. For the subsequent experiments the mosquitoes used were reared from eggs obtained at two localities in Guatemala, El Salto in the Department of Esquintla and El Remate in the Department of Peten. Large numbers of *equinus* eggs had been obtained at these localities in the course of collecting the material of *mesodentatus mesodentatus* and *mesodentatus gorgasi*, forms in which we were primarily interested.

The *Haemagogus mesodentatus mesodentatus* and *mesodentatus gorgasi* were reared from eggs obtained respectively at El Remate and El Salto, Guatemala.

The material of *Haemagogus lucifer* was obtained at Cerro La Victoria, Panama. As this species was not colonized and the number of eggs gotten from wild caught females was not large, only limited numbers were available and this species was used only in the first and third experiments.

A colony of *Sabethes chloropterus* had been established by the time the second and third experiments were performed. The wild stock had been obtained at Cerro La Victoria, Panama. In the second experiment both colony-reared individuals and wild caught material, also from La Victoria, was used, while in the third experiment all the *chloropterus* were colony stock. Insofar as we know this is the first time a sabethine mosquito has been colonized. While the species with which we worked lives well and mates readily in captivity, great difficulty was experienced in getting them to feed in the laboratory, although they attack freely in the field. It was particularly difficult to obtain refeeding.

METHODS

For feeding the mosquitoes several methods were used. Usually the *Haemagogus* were first put in Barraud cages, eight inches square, and the shaved foot of the monkey inserted. When adequate feeding was not obtained by this method the mosquitoes were transferred to one-pint cylindrical cardboard cartons which had been provided with netting at either end. The cartons were then applied to the shaved belly of the monkey. Mosquitoes which had not fed by either of these methods were transferred individually to shell vials and these were then put on the shaved belly of the monkey. The *Sabethes chloropterus*, which in the field bite primarily about the head and similarly fed in the laboratory, could not be induced to bite by the methods used for the *Haemagogus*. They were released in a cage three feet long, two feet wide and 18 inches high, and the entire body of the trussed monkey was put in the cage. Under these conditions a portion of the specimens would feed on the face of the monkey when he did not move too much, and there was also some feeding on the hands.

On the day before feeding experiments were scheduled, food (sugar-soaked cotton balls) was removed from the stock cages. After feeding, all mosquitoes were placed in one-pint cardboard cartons provided with netting at both ends, and these were then held on a shallow pan covered with moistened cotton and filter paper. Balls of sugar-soaked cotton were kept on the tops of the cartons at all times until the day before refeeding was attempted. As there was an abundant growth of mould on the feces excreted in the days after the first feeding, the mosquitoes were usually transferred to fresh cartons after about a week.

In the first experiment the mosquitoes were held in an outdoor insectary where the daily range in temperature was from 72°F. to 90°F. and the bi-hourly mean was 78°F. For the second and third experiments an enclosed insectary was available in which the temperatures ranged from 76°F. to 85°F. and the bi-hourly mean was 79°F.

Mosquitoes were allowed to engorge on monkeys on the 2nd to 5th day after subcutaneous injection with yellow fever virus. Immediately prior to exposure to the bites of the mosquitoes, blood was drawn from the femoral veins of the monkeys and the serum diluted serially in 10 per cent inactivated, filtered, negative rhesus serum-saline for titration in mice. Six mice were injected intracerebrally with each decimal dilution. At the end of the 30-day observation period surviving mice were challenged for immunity by the intracerebral injection of between 5,000 and 10,000 LD/50 of the French neurotropic yellow fever virus. As the Trinidad strain of virus isolated from the spleen of a howling monkey showed relatively low pathogenicity for mice, the minimal infective titers (ID/50) obtained may not represent accurately actual virus titers.

The mosquitoes were maintained for an incubation period of 22 to 44 days. Any found dead or dying during the first five days after engorgement were discarded. Those found recently dead or dying subsequent to the first five days were triturated in 10 per cent rhesus serum-saline for injection into mice, either singly or in small pools of 2 to 4 mosquitoes. Mice were kept under observation for 30 days. Those surviving this period were challenged for immunity by the intracerebral injection of 5,000 to 10,000 LD/50 of French neurotropic yellow fever virus.

At the end of the incubation period the surviving mosquitoes of each species were allowed to bite a normal rhesus or spider monkey. Temperatures of the monkeys were taken twice daily, morning and afternoon. The animals were bled every other day and the serum injected intracerebrally into mice to detect circulating virus. Pathological study of the livers of monkeys dying of the infection was made by Dr. Carl M. Johnson who in each instance confirmed the diagnosis of yellow fever. Surviving monkeys were bled at three, four and six-week intervals and their serum tested for mouse protecting antibodies. Mosquitoes which probed or engorged on the test monkeys were emulsified with serum-saline and injected into mice.

Identification of virus. Mice developing suggestive symptoms during the 30-day observation period were sacrificed. Impression smears stained by the method of Giemsa and cultures in blood agar plates and in thioglycollate broth were made from their brain tissue.

Virus isolated in mice from the blood of monkeys bitten by infected mosquitoes was identified by cross-immunity and protection tests. For the former a group of normal mice and a group of mice previously immunized against the French neurotropic yellow fever virus were injected intracerebrally with first mouse passage virus. Results were considered positive if the immunes were alive and well after the non-immunes had sickened or died. In the protection tests the intraperitoneal technique was employed combining mouse passage virus with known positive and negative monkey serums.

Due to the very large number of isolations of virus from mosquitoes feeding on infected monkeys, protection tests were not done. In view of the known history of these mosquitoes, identification of yellow fever virus was considered established when bacterially negative smears and cultures were obtained and cross-immunity experiments gave results as previously described.

The experiments were performed in three groups. The general technique previously described was followed in each instance.

EXPERIMENT 1

In the first experiment (see Table 1) the virus strain isolated in mice from specimen # 4754, serum of a nonfatal human case of yellow fever, was used. This virus had been passaged three times in mice and once in a rhesus monkey prior to subcutaneous injection of the rhesus monkey used for infecting the mosquitoes. Mosquitoes used were *Haemagogus mesodentatus gorgasi, spegazzinii falco, lucifer* and two strains of *equinus*, one from Panama and one from Guatemala. The mosquitoes were fed on the rhesus monkey on the second, third and/or fourth days after inoculation. Virus titers were low, the highest being $10^{-2.5}$ on the second day after inoculation.

Only *H. mesodentatus gorgasi* and *spegazzinii falco* survived long enough to be used for bite transmission attempts, each species being allowed to engorge

TABLE 1

First transmission experiment with a Trinidad strain of yellow fever virus isolated from human serum (specimen # 4754)

Species of Mosquito	Day of Feeding	Titer of Circulating Virus ID/50	Results of Mouse Injections			Results of Refeeding on Monkeys		
			Total no. mosquitoes injected	No. virus isolations	Per cent positive (Min. & Max. possible)	No. of mosquitoes biting	Incubation period (days)	Outcome
Haemagogus mesodentatus gorgasi	2nd	$10^{-2.5}$	16	2	12.5–37.5			
	3rd	$10^{-1.8}$	2	0				
	2nd & 3rd (pool)	$10^{-2.5}$ & $10^{-1.8}$	18	1	5.6	12*	29–33	Negative
Haemagogus spegazzinii falco	2nd	$10^{-2.5}$	3	0		1	31	Negative
	3rd	$10^{-1.8}$	10	2	16.6	6†	28–32	
Haemagogus lucifer	2nd	$10^{-2.5}$	3	0				
	3rd	$10^{-1.8}$	3	0				
	4th	$<10^{-1}$	2	0				
Haemagogus equinus (Panama)	2nd	$10^{-2.5}$	10	0				
Haemagogus equinus (Guatemala)	2nd	$10^{-2.5}$	17	1	4.2–12.5			
	3rd	$10^{-1.8}$	7					

* Subsequent injection of mice with suspensions of individual mosquitoes gave one positive.

† Subsequent injection of mice with suspensions of individual mosquitoes gave two positives.

on a normal rhesus monkey. *Haemagogus mesodentatus gorgasi* which had fed on the second and third days were put together for refeeding in the transmission attempt. Results are tabulated in Table 1. No transmission by bite was obtained, which was to be expected in view of the low virus titers in the infecting monkey. Neither monkey showed fever or other symptoms, circulating virus or subsequent immunity. However, this experiment was not without interest because virus was recovered from a small proportion of the mosquitoes by injection of mice, in spite of the low titers in the circulating blood. Of the *H. spegazzinii falco* which had engorged on the third day, when the virus titer was $10^{-1.8}$, 16.6 per cent were positive. The exact proportion of positive *H. mesodentatus gorgasi* could not be determined since pools of mosquitoes were used in several instances. However, those engorging on the second day, when the circulating virus titer was $10^{-2.5}$, showed a proportion of positives somewhere between 12.5 and 37.5 per cent depending on whether only one or all of the mosquitoes in the positive pools contained virus.

EXPERIMENT 2

For the second transmission experiment (see Table 2) the strain of yellow fever virus isolated from the spleen of a howling monkey, specimen ✻4204, was used. Three monkeys, two rhesus and one spider monkey, were injected subcutaneously with this strain in its first experimental monkey passage; i.e., with serum of a rhesus injected with the original *Alouatta* spleen suspension. The following species of mosquitoes were employed: *Haemagogus mesodentatus gorgasi*, *mesodentatus mesodentatus*, *Sabethes chloropterus* and two strains of *H. equinus*, from El Remate and El Salto, Guatemala. (The *Haemagogus equinus* from El Remate died in less than 26 days and therefore were not used for bite transmission.) After an incubation period of 26 to 34 days each species was allowed to bite a normal rhesus monkey. Transmission by bite was obtained in each instance. The rhesus monkeys bitten by the three *Haemagogus* forms all developed fever in three to five days after the first day of refeeding and died in six to seven days. It is of interest that the rhesus bitten by only one *H. mesodentatus mesodentatus* suffered a rapidly fatal infection. The monkey (*Macaca mulatta*) on which *Sabethes chloropterus* was refed was exposed each of five days. It developed fever 15 days after the first exposure and ten days after the last, and died two days later. This prolonged incubation period may indicate infection with a minimal amount of virus. Virus was isolated, by injection of mice, from a high percentage of the *Haemagogus* mosquitoes (see Table 2). Virus was not obtained from any of the 49 *Sabethes chloropterus* injected into mice after periods of incubation varying from 12 to 44 days. The discrepancy between successful transmission by bite and unsuccessful isolation by injection must be resolved in future experimentation.

EXPERIMENT 3

The strain of virus obtained from *Alouatta* spleen was again used in the third experiment. It had been passaged twice in rhesus monkeys. The following mosquitoes were used in this experiment: *Haemagogus mesodentatus gorgasi*, *lucifer*,

TABLE 2

Second transmission experiment with a Trinidad strain isolated from Alouatta *spleen (specimen # 4202)*

Species of Mosquito	Day of Feeding	Titer of Circulating Virus ID/50	Results of Mouse Injections			Results of Refeeding on Monkeys		
			Total no. mosquitoes injected	No. virus isolations	Per cent positive (Min. & Max. possible)	No. of mosquitoes biting	Incubation period (days)	Outcome
Haemagogus mesodentatus gorgasi	3rd (Rh #1)	$10^{-5.2}$	2	2	100	4	26–28	Positive
	4th (Rh #1)	$10^{-5.3}$	0			2		
	3rd & 4th (pool)	$10^{-5.3}$ & $10^{-5.3}$	8	5	62.5			
Haemagogus mesodentatus mesodendatus	3rd (Rh #1)	$10^{-5.2}$	0			1	27	Positive
	4th (Rh #1)	$10^{-5.3}$	3	2	66.7			
	3rd (Rh #2)	$10^{-5.4}$	16	2	12.5–50			Positive
Sabethes chloropterus	3rd (Rh #1)	$10^{-5.2}$	4	0		7	27–34	Positive
	4th (Rh #1)	$10^{-5.3}$	4	0		2(4)*		
	3rd (Rh #2)	$10^{-5.4}$	10	0		0(1)		
	3rd (RS #1)	$10^{-5.8}$	1	0		2		
	pool of the above		30	0				
Haemagogus equinus (Peten, Guatemala)	3rd (Rh #1)	$10^{-5.2}$	1	1	100	0		
	3rd (Rh #2)	$10^{-5.4}$	20	7	35–80	0		
Haemagogus equinus (El Salto, Guatemala)	3rd (Rh #1)	$10^{-5.2}$	68	30	44–70	10	26–28	Positive
	4th (Rh #1)	$10^{-5.2}$	4	0		16		

* Parentheses indicate specimens which probed but did not feed. Rh = Rhesus (*Macaca mulatta*). RS = Red spider monkey (*Ateles geoffroyi*).

Sabethes chloropterus and *H. equinus*, the last from El Salto, Guatemala. All species were fed on a single rhesus monkey on the third, fourth and fifth days after injection when the circulating virus titers were $10^{-5.8}$, $10^{-4.2}$, and $10^{-3.8}$ respectively.

After an incubation period varying from 22 to 29 days, surviving mosquitoes were allowed to feed on normal monkeys. *Haemagogus equinus, lucifer* and *Sabethes chloropterus* were each fed on rhesus monkeys. *H. mesodentatus gorgasi* was fed on a black spider monkey. An infection fatal in seven days was produced in the rhesus bitten by *H. equinus*. The spider monkey on which *H. mesodentatus gorgasi* was fed did not die but showed circulating virus and was subsequently immune. Only one *H. lucifer* attempted to feed on the rhesus monkey. It is uncertain whether it simply probed or actually fed. In either case results were negative.

In this experiment only the *H. lucifer* and *S. chloropterus* were injected into mice as it was thought that sufficient data had already been accumulated on the other two species. The exact percentage of *H. lucifer* positive by mouse injection could not be determined due to the use of some pooled specimens. However,

TABLE 3

Third transmission experiment with a Trinidad strain isolated from Alouatta *spleen (specimen #4204)*

Species of Mosquito	Day of Feeding	Titer of Circulating Virus ID/50	Results of Mouse Injections			Results of Refeeding on Monkeys		
			Total no. mosquitoes injected	No. virus isolations	Per cent positive (Min. & Max. possible)	No. of mosquitoes biting	Incubation period (days)	Outcome
Haemagogus mesodentatus gorgasi	3rd	$10^{-5.8}$	0			5(11)*	27–29	Positive
	4th	$10^{-4.2}$	0					
	5th	$10^{-3.8}$	0					
Haemagogus lucifer	3rd	$10^{-5.8}$	32	11	34–59	1†		Negative
	4th	$10^{-4.2}$	4	0				
	5th	$10^{-3.8}$	10	0				
	pool of the above		4	1	25–50			Negative
Sabethes chloropterus	3rd	$10^{-5.8}$	9	0		3	27–29	Negative
	4th	$10^{-4.2}$	0					
	5th	$10^{-3.8}$	0					
Haemagogus equinus (El Salto, Guatemala)	3rd	$10^{-5.8}$	0			3–5	22–24	Positive
	4th	$10^{-4.2}$						
	5th	$10^{-3.8}$						

* Parentheses indicate specimens which probed but did not feed.
† Uncertain whether probed or fed.

of those originally fed on the monkey on the third day when the circulating titer was $10^{-5.8}$, between 34 and 59 per cent were positive by mouse injection.

No virus isolations in mice were obtained in this experiment with any of nine *S. chloropterus*, all fed on the third day, nor was transmission by bite obtained.

DISCUSSION

In view of the many uncontrollable variables in an experiment of this kind, it is difficult to make an accurate interpretation of all results obtained. The principal objectives, however, were achieved. Fair to high proportions of all *Haemagogus* species tested harbored virus. The exact proportion varied with the titer of virus in the infective meal. Successful transmission by bite was obtained twice in three trials with *H. mesodentatus gorgasi*, twice in two trials with *H. equinus* and once in the trial made with *H. mesodentatus mesodentatus*. The failure of *H. mesodentatus gorgasi* to transmit by bite in the first experiment may be attributed to use of a mouse-passaged strain of yellow fever virus which developed only very low titers in the blood of the source monkey. The direct correlation between virus titer in the infective meal and subsequent capacity to transmit by bite has been amply demonstrated by Waddell and Taylor, 1947, and Bates and Roca-Garcia, 1946, among others. It is significant that under equal circumstances, the control species, *H. spegazzinii falco*, also failed to transmit by bite, although two of the seven engorging mosquitoes were positive by mouse injection.

The experiment involving bite transmission by *H. lucifer* must be considered unsatisfactory as only one mosquito bit or probed.

No *Sabethes chloropterus* were found to harbor virus, at least in sufficient amounts to infect mice. No mouse injected with suspensions of this species showed symptoms or developed a subsequent immunity. One experimental transmission by bite was obtained in two attempts. In the successful transmission experiment, 11 mosquitoes fed and five probed. In the unsuccessful experiment, only three fed. The interpretation of this result is difficult and further experimentation is required.

SUMMARY

Successful transmission from monkey to monkey of a strain of yellow fever virus isolated in Trinidad was obtained with the following Middle American *Haemagogus: H. m. mesodentatus, H. m. gorgasi* and *H. equinus*. All the above mentioned species as well as *H. lucifer* and *H. spegazzinii falco* were found by intracerebral mouse inoculation to harbor yellow fever virus from one to four weeks after the infective meal. No virus was isolated from mice injected with pools of *S. chloropterus* even though the species transmitted the virus by bite from monkey to monkey on one occasion.

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STUDIES OF THE SINDBIS VIRUS. PART I. SUSCEPTIBILITY OF THE CAVE BAT BY VARIOUS ROUTES OF EXPOSURE

REGINALD L. REAGAN, FRANCES S. YANCEY, SING CHEN CHANG AND A. L. BRUECKNER

Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland

The present study was undertaken to determine susceptibility of cave bats (*Myotis lucifugus*) to Sindbis tropical virus. The Sindbis virus was first encountered in August of 1952 in a group of *Culex pipiens* or *Culex univittatis* mosquitoes captured by light traps in the Sindbis (Egypt) health district located several kilometers north of Cairo. The isolation was made by inoculation of a suspension of triturated mosquitoes into 3-day old suckling mice. The virus is highly pathogenic to embryonating chicken eggs. Seven-day-old embryos exhibit generalized congestion, petechial hemorrhages of the integument and membrane and die within 24 to 72 hours after yolk sac inoculation with dilutions up to 10^{-7}. Embryos infected via the allantoic sac do not have allantoic fluid containing agglutinins for chicken erythrocytes and serial passage of this virus cannot be carried successfully in adult mice or hamsters but the suckling mouse is highly susceptible (Taylor *et al.*, 1955).

MATERIALS AND METHODS

Sindbis virus was obtained from the American Type Culture Collection in Washington, D.C., a public health permit being required for work with this virus. The virus, received in the lyophilized state, was reconstituted with 2 ml. of physiological saline and injected via the yolk sac into 12 seven-day-old embryonating chicken eggs. Upon death of the embryos (48 to 72 hours later) the infected allantoic-amniotic fluid was harvested, pooled and stored in the deep freeze vault at −60C. before initiating the experiment. Culture of the allantoic-amniotic suspension showed no bacterial growth.

Thrity-six healthy cave bats were divided into six groups. Each of five bat groups was exposed to the Sindbis virus by one of the following routes: intracerebral, intraperitoneal, intradermal, intrarectal or intranasal. The inoculum consisted of 0.03 ml. of the infected allantoic-amniotic fluid. The sixth group was held as a room control and received no virus. Each group was kept in a special glass jar with paper towels for clinging and was fed a diet of a mixture of banana, cottage cheese, condensed milk and Pablum. They were given plenty of water daily. After inoculation, all groups were observed twice daily for nervous symptoms characteristically found in mammals infected with this virus. After symptoms of nervous system involvement appeared, the bats were sacrificed. The brains were removed aseptically from each group including the normal control, pooled and stored at −60 C. in a deep freeze vault (Table 1). Later, they were removed from the deep freeze vault and after trituration diluted to a

TABLE 1

Response of cave bats (Myotis lucifugus) *to Sindbis virus*

Route of Inoculation	No. of Rats Exposed	No. Showing Symptoms
Intraperitoneal	6	6 (3rd to 6th day)
Intrarectal	6	6 (3rd to 6th day)
Intradermal	6	6 (4th to 6th day)
Intranasal	6	6 (3rd to 5th day)
Intracerebral	6	6 (3rd to 4th day)
Control	6	None

TABLE 2

Neutralization tests conducted in 7-day-old embryonating chicken eggs

Suspension	Neutralization Result using Anti-Sindbis Hamster Immune Serum
Intraperitoneal brain suspension (pool: 6 bats)	Positive for Sindbis virus
Intrarectal brain suspension (pool: 6 bats)	Positive for Sindbis virus
Intradermal brain suspension (pool: 6 bats)	Positive for Sindbis virus
Intranasal brain suspension (pool: 6 bats)	Positive for Sindbis virus
Intracerebral brain suspension (pool: 6 bats)	Positive for Sindbis virus
Control brain suspension (pool: 6 bats)	Negative for Sindbis virus

20 per cent suspension with physiological saline. These suspensions were tested for Sindbis virus by neutralization tests (Table 2) conducted in seven-day-old embryonating eggs using immune serum prepared by immunizing adult hamsters. Table 1 shows the response of cave bats to Sindbis virus by different routes of inoculation.

SUMMARY

Cave bats (*Myotis lucifugus*) are susceptible to the Sindbis virus by the intraperitoneal, intrarectal, intranasal and intracerebral routes of exposure. The normal control bats used as room and food controls appeared normal throughout the experiment.

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STUDIES OF THE SINDBIS VIRUS. PART II. SUSCEPTIBILITY OF THE SUCKLING HAMSTER BY VARIOUS ROUTES OF EXPOSURE

REGINALD L. REAGAN, FRANCES S. YANCEY, SING CHEN CHANG AND A. L. BRUECKNER

Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland

The present study was undertaken to determine susceptibility of suckling hamsters to Sindbis tropical virus by various routes of exposure. Sindbis virus was first encountered in August of 1952 in a group of 63 *Culex pipiens* or *Culex univittatus* mosquitoes captured by light traps in the Sindbis (Egypt) health district located several kilometers north of Cairo. The isolation was made by inoculation of a suspension of triturated mosquitoes into 3-day old suckling mice. The virus is highly pathogenic to embryonating eggs. Seven-day-old embryos exhibit generalized congestion, petechial hemorrhages of the integument and membrane and die within 24 to 72 hours after yolk sac inoculation with dilutions up to 10^{-7}. Embryos infected via the allantoic sac do not have allantoic fluid containing agglutinins for chicken erythrocytes and serial passage of this virus cannot be carried successfully in adult mice or hamsters, but the suckling mouse is highly susceptible (Taylor *et al.*, 1955).

METHODS AND RESULTS

Sindbis virus was obtained from the American Type Culture Collection in Washington, D. C., a public health permit being required for work with this virus. The virus, received in the lyophilized state, was reconstituted with 2 ml of physiological saline and injected via the yolk sac into 12 seven-day-old embryonating chicken eggs. Upon death of the embryos (48 to 72 hours later), the infected allantoic-amniotic fluid was harvested, pooled and stored in the deep freeze vault at −60 C. before initiating the experiment. Culture of the allantoic-amniotic virus suspension showed no bacterial growth.

Five hamster mothers with sucklings were placed in special metal boxes with bedding. The sucklings were exposed to the Sindbis virus by each of the following routes: intracerebral, intraperitoneal, intradermal, intrarectal or intranasal. The inoculum consisted of 0.03 ml. of the infected allantoic-amniotic fluid. The sixth group was held as a room control and received no virus. The mother received no virus but was used only for nutrition of the sucklings. Each group was given plenty of water daily and the mother was fed dog checkers. After inoculation, all groups were observed twice daily for nervous symptoms characteristically found in mammals infected with this virus. The mothers showed no signs of Sindbis disease and were discarded after a 21-day observation period.

After symptoms of nervous system involvement appeared, the sucklings were sacrificed. The brains were removed aseptically from each group including the normal control, pooled, and stored at −60 C. as shown in Table 1. Later, they

TABLE 1

Response of suckling hamsters to Sindbis virus

Route of Inoculation	No. of Sucklings Exposed	No. Showing Symptoms
Intraperitoneal	6	6 (3rd to 5th day)
Intrarectal	5	5 (3rd to 6th day)
Intradermal	6	6 (4th to 7th day)
Intranasal	7	7 (3rd to 5th day)
Intracerebral	6	6 (3rd to 4th day)
Control	6	None

TABLE 2

Neutralization tests conducted in 7-day-old embryonating chicken eggs

Suspension	Neutralization Result Using Anti-Sindbis Hamster Immune Sera
Intraperitoneal brain suspension (pool: 6 suckling hamsters)	Positive for Sindbis virus
Intrarectal brain suspension (pool: 6 suckling hamsters)	Positive for Sindbis virus
Intradermal brain suspension (pool: 6 suckling hamsters)	Positive for Sindbis virus
Intranasal brain suspension (pool: 6 suckling hamsters)	Positive for Sindbis virus
Intracerebral brain suspension (pool: 6 suckling hamsters)	Positive for Sindbis virus
Control brain suspension (pool: 6 suckling hamsters)	Negative for Sindbis virus

were removed from the deep freeze vault and after trituration diluted to a 20 per cent suspension with physiological saline. These suspensions were tested for Sindbis virus by neutralization tests (Table 2) in seven-day-old embryonating chicken eggs using immune serum prepared by immunizing adult hamsters. Table 1 shows the response of the sucklings to Sindbis virus by different routes of inoculation.

SUMMARY

Suckling hamsters are susceptible to the Sindbis virus by the intraperitoneal, intrarectal, intradermal, intranasal and intracerebral routes of exposure. The normal control sucklings used as room and food control appeared normal throughout the experiment. The virus in the suckling hamster brain was confirmed to be Sindbis virus by neutralization tests conducted in embryonating chicken eggs.

REFERENCE

TAYLOR, R. M., HURLBUT, H. S., WORK, T. H., KINGSTON, J. R., AND FROTHINGHAM, T. E., 1955. Sindbis virus: a newly recognized arthropod-transmitted virus, *Am. J. Trop. Med. & Hyg.* **4**: 844–862.

SOME ASPECTS OF YAWS IN LIBERIA

F. S. DA CRUZ-FERREIRA[1] AND H. STERENBERG

Liberia has an area of about 40–50 thousand square miles and the estimated population is about 1.5 million people. It is roughly estimated that there are some 600,000 cases of yaws in Liberia and thus yaws at the present time is one of the most important problems to be considered in programs of public health in that country.

Although surveys have not included systematic parasitological examination, but have been based on clinical evidence of disease, it is possible to say that yaws is in fact the most important disease from the clinical point of view and, after malaria, the most imperative public health problem. With natural differences of prevalence between the coastal zones and the hinterland, and in areas more or less distant from the motor roads, it can be said that about 50 per cent of the population show signs of yaws (10,472 cases in 20,619 persons examined). This estimate is based principally on clinical cases (infectious or late cases) because, on account of the difficulty of getting accurate information from the people, the diagnosis of latent cases was made chiefly on the grounds of typical sequelae with a very clear history attributable to the disease. Everybody wishing an injection during the surveys will automatically give a history of yaws. Taking into account the latent cases, which serological studies have proved to be important, the figure of general prevalence of the disease must be stepped up to about 70 per cent. Of 757 persons without clinical signs of yaws who were tested by the Venereal Disease Research Laboratory procedure (VDRL), 46.6 per cent proved to be reactive, many of them to the higher dilutions.

This paper gives information about epidemiological, clinical and serological findings it was found possible to obtain through surveys in the hinterland from August, 1953, to March, 1954, in the area of a pilot project in Liberia.

The chief aims of the pilot project were to investigate as far as possible the prevalence and gravity of yaws; to obtain knowledge of its natural history and epidemiology; and by clinical and serological follow-up, to evaluate the results of two schemes of therapy with a view to the eradication of the disease by a mass treatment campaign. It was kept in mind that the work, though planned with a practical objective, would have to take advantage of all the possibilities of research in order to clear up some unknowns in connection with the control of the disease.

The areas of survey were selected in the hinterland of Saniquelli and Gbarnga districts, and four methods were followed. 1) In villages on the motor roads, only clinical cases were treated. 2) In the Bahn area, cases were treated, and contacts (children up to ten years of age) were given protective doses. The villages were situated roughly up to ten miles from the farthest center accessible by car. 3) In the Saclepea area all cases were treated, and contacts (children and adolescents up to 18 years of age) were protected. 4) In the Gbarnga area all

[1] World Health Organization, P. O. Box 6, Brazzaville, French Equatorial Africa.

cases were treated, and the rest of the population considered as contacts and given protective doses.

This schedule was adopted in order to provide epidemiological data on the prevalence of yaws after different classes of contacts had been protected. Simultaneously it was possible to obtain further information by carrying out special clinical and serological research to show: (a) differences in the prevalence of infectious cases in relation to certain environmental conditions, and the occurrence of yaws according to the sex, age and type of lesion observed; (b) serological results found in different types of yaws lesions according to the latest classification recommended by WHO; (c) percentage of reactors among Africans without clinical signs of yaws, and the differences of reagin titers in treated and untreated cases in the same villages.

It is premature at the time of writing to assess the modifications of the reagin titers of patients under the influence of the treatment, which was an important objective of the work. Follow-up surveys were started in March, 1954, but clinical data have been obtained with great difficulty. Future experience will show the practical possibilities of such a follow-up.

In patients attending the Kpain clinic it was also possible to confirm the percentage of infectious cases by darkfield examination.

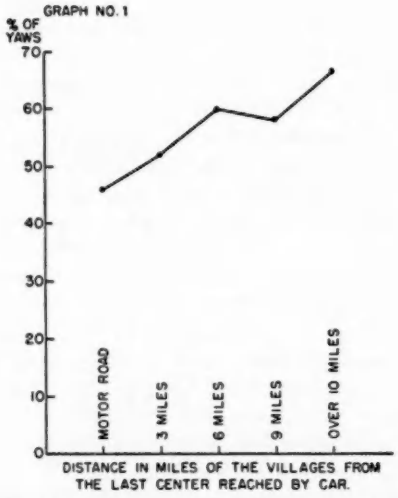
EPIDEMIOLOGICAL ASPECTS

1. *Incidence of yaws in relation to the distance of villages from the main road.* The percentage of yaws in an endemic area, as is well known, is lower in the villages along the roads and increases, in bush areas, with the difficulty of communication. Only in isolated villages situated on the tops of hills, not surrounded by heavy vegetation, well-exposed to the wind and with rocky soil was this situation not found. In such places there was a relatively low percentage of yaws.

The incidence of yaws was evaluated in groups of villages only accessible on foot and at about the same distance from the last center which could be reached by car. The analysis of the prevalence of yaws in relation to this distance is shown in Graph 1.

Roughly it can be said that the prevalence of yaws increases in direct proportion to the distance of the villages from the main motor roads. It has already been said that "yaws is a disease of the end of the road" and these data seem to confirm the truth of this statement.

2. *Incidence of yaws according to some meteorological conditions.* As far as possible, the meteorological and environmental conditions in the center of the main area of operations were recorded in order to evaluate the relationship between these variables and the prevalence of infectious cases. Meteorological data taken at Kpain, as well as the calculated effective temperatures, are shown in Table 1. From these data it seems that the percentage of infectious cases has a definite relationship to the intensity of the rains. As a tentative conclusion it appears that there is a correlation in time between rainfall and the incidence of infectious yaws, with a time-lag of 1–2 months for the latter. Further data with respect to the other months of the year will be necessary to substantiate this.



GRAPH No. 1. Prevalence of clinical yaws in relation to the distance of villages from the motor road.

TABLE 1

The general meteorological conditions in the center of the main area of operations (Kpain, Liberia, West Africa)

Altitude of Kpain—1,400 feet

	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Temp. (°F.)								
Max.	78.6°	81.9°	83.3°	84.5°	84.1°	84.6°	86.3°	84.6°
Min.	71.9°	73.2°	73.8°	74.2°	74.3°	73.6°	75.0°	74.0°
Rel. humidity* (average)	90.8	86.0	84.6	81.8	79.6	78.4	73.8	80.7
Wind speed†	0.6–5.2	0.6–12.4	0.6–5.2	0.6–7.40	0.6–5.2	0.6–7.4	0.6–12.4	—
Rainfall (mm.)	267.10	303.50	192.3	52.0	2.90	23.50	99.50	209.60
Effective temp.‡	68.5°	69.0°	73.0°	73.5°	74.5°	73.5°	71.5°	74.5°
% of infectious cases	8.5	9.5	5.8	8.9	3.7	2.8	3.1	3.0

* Relative humidity calculated according to the tables of the U. S. Weather Office, No. 1071.

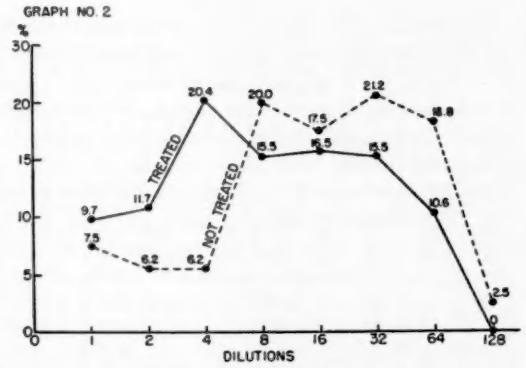
† Wind velocity according to the Beaufort International Scale.

‡ Effective temperature in degrees F. calculated on thermometrical graph from the American Society of Heating and Ventilation Engineers, Guide 1932. Averages were taken monthly from a daily observation of maximum and minimum temperatures.

Dry and wet bulb temperature, wind velocity and direction, rain-fall and cloud index as at 8 a.m., 2 p.m., and 8 p.m.

On the other hand, no definite relationship between the percentage of infectious cases and other registered variables of the climate was found.

3. *Differences of reagin titers between patients treated and not treated, in the same villages.* It was found that serological surveys in villages near the centers of



GRAPH No. 2. Percentage distribution of treated and untreated patients according to reagin titers.

TABLE 2

Prevalence of clinical yaws by months, as determined by field surveys

Month (1953–1954)	No. Examined	Clinical Yaws					
		Total		Infectious		Late	
		No.	%	No.	%	No.	%
August	1,329	890	66.9	113	8.5	777	58.4
September	3,589	2,255	62.8	340	9.5	1,915	53.3
October	2,726	1,510	55.4	159	5.8	1,351	49.6
November	2,805	1,466	52.2	249	8.9	1,217	43.3
December	4,396	2,219	50.4	164	3.7	2,055	46.7
January	2,738	992	36.2	76	2.8	916	33.4
February	2,505	932	37.2	77	3.1	855	34.1
March	531	208	39.2	16	3.0	192	36.2
Total	20,619	10,472	50.7	1,194	5.8	9,278	44.9

treatment showed a percentage of non-reactors greater than that in the hinterland. However, the titers found in quantitative tests of the reactors were nearly as high as those registered in the other areas.

A group of four villages was re-surveyed and 221 persons were tested (127 yaws patients treated with PAM in the first survey and 94 new patients, absentees during the first surveys and not treated). The results of the VDRL tests showed 103 (81.1%) of the treated and 80 (85.1%) of the untreated to be reactive. The results in percentages obtained in quantitative tests are indicated in Graph 2.

CLINICAL ASPECTS

The first field surveys were carried out from August, 1953, to March, 1954, with the results indicated in Table 2.

The distribution by age group, sex and type of lesion of 8,892 cases is given in Table 3.

TABLE 3

Distribution of yaws by age group, sex, and type of lesion

Age Groups	Sex	Type of Lesion*										Total (Sex)	Total (Age Groups)
		1	2	3	4	5	6a	6b	7	8	9		
Under 2	M	2	13	—	7	—	1	—	3	—	—	26	59
	F	—	21	2	5	—	3	—	2	—	—	33	
2-10	M	20	331	84	46	484	52	—	59	12	3	1,091	1,947
	F	28	222	68	60	372	50	1	39	14	2	856	
11-18	M	1	17	31	10	377	21	—	30	14	1	502	1,148
	F	—	13	35	8	526	27	—	28	3	6	646	
19 over	M	—	3	7	2	2,048	25	5	69	79	248	2,486	5,738
	F	1	4	18	14	2,506	48	10	82	66	503	3,252	
Total	No.	52	624	245	152	6,313	227	16	312	188	763	(M) 4,105	8,892
	%	0.6	7.0	2.8	1.7	71.0	2.6	0.2	3.4	2.1	8.6	(F) 4,787	

* 1. Initial lesion; 2. Multiple papillomata; 3. Wet crab; 4. Other early skin lesions; 5. Hyperkeratosis; 6a. Gummata and ulcers; 6b. Gangosa; 7. Bone and joint lesions; 8. Latent cases; 9. Other manifestations.

Observed frequency of types of lesion. The classification of lesions used is in accordance with the international nomenclature of yaws adopted by the Fourth Session of the WHO Expert Committee on Venereal Infections and Treponematoses, comprising nine types of lesion as shown in Table 3.

A certain percentage of patients presented a combination of lesions at the time of examination. The more important or predominant type of lesion was recorded as the diagnosis and priority was given to infectious cases. Generally speaking, the group of lesions 1, 2, 3, and 4 was more frequent in children between 2–10 years of age, and of these the most important was the multiple papilloma, followed by wet crab, other early skin lesions and to a minor degree the initial lesion. Hyperkeratosis was the most common lesion observed at all ages.

1. *Initial lesion.* This was rarely observed. It was more common to see patients with multiple papillomata already developed, and among such patients it was very difficult to find the initial lesion, which was sometimes in the healing stage. The initial lesion was observed principally in children between 2–10 years of age. Only one adult (a female over 19 years of age) and a male (between 11 and 18 years of age) and two infants (males under 2 years) were recorded as having initial lesions.

The most frequent lesion observed was a giant papilloma showing some protuberance from the skin, sometimes starting to heal. Less often a papuloulcerous lesion was seen. An early crateriform lesion without indurated base and surrounded by a hypopigmented area was considered an initial lesion.

2. *Multiple papillomata.* After hyperkeratosis and "other manifestations", these were the most frequent lesions observed. They were the most typical lesions

of yaws in children (553 cases in both sexes between 2–10 years of age). Seven cases of multiple papillomata were seen in adults (3 males and 4 females). The number of papillomata present varied from 2 or 3 to a generalized eruption covering the whole body (including the scalp) and in various stages of evolution, some healing, others with the typical exudate and yellow granulation.

3. *Wet crab.* This was considered the most painful lesion of yaws, interfering seriously with the walking and working of the patients. Almost as painful were the early bone and joint lesions, and plantar hyperkeratosis.

4. *Other early skin lesions.* The cases recorded were of several types, the circinate lesions and the micro- or macro-papular eruption being the most common, generalized or in patches, and involuting with desquamation. We found also some maculoerythematous rashes, with or without dyschromia, and furfuraceous desquamation in well defined patches with a powdery appearance. The differential diagnosis between early skin lesions of yaws and lesions of other skin diseases is among the most difficult in tropical Africa. Some cases considered clinically typical of yaws were found to be non-reactive when serologically examined.

5. *Hyperkeratosis.* The plantar and palmar hyperkeratoses, with or without other manifestations of yaws, were the most frequent lesions. Their frequency increased with age, being 51.2 per cent in adults, 10.2 per cent in the age group 11–18 years and 9.6 per cent in the age group 2–10 years. In children the simultaneous occurrence of hyperkeratosis, multiple papillomata and bone lesions was sometimes recorded. Plantar hyperkeratosis was more frequent than palmar. The plantar hyperkeratosis was often ulcerated, deeply fissured or eroded, and very painful. In the palmar form, desquamation of the skin and pigmentary changes were more frequent. Leucoderma was sometimes seen, and leuco-melanoderma involving the foot or the hand, reaching the dorsal side. Palmar hyperkeratosis sometimes was associated with tenosynovitis which was most often found in the little finger. In this group of lesions it is quite possible that some diagnostic mistakes were made, owing to the difficulty of differential diagnosis resulting from repeated trauma, fissures caused by implements, fungoid diseases, etc., that can cause confusion. However, a great deal of attention was given to these possibilities and many were tested serologically.

6. *Gummata and ulcers.* Gummata and typical ulcers were more frequent in children between 2–10 years of age. The ulcers are painless, with sharp edges and sometimes with partial cicatrization of the surrounding skin, which can be atrophic or depigmented.

7. *Gangosa.* Gangosa was practically confined to adults. Only one case was seen in a girl of the age group 2–10 years.

8. *Bone and joint lesions.* Bone and joint lesions were more common in adults, followed by the age group 2–10 years and adolescents. Bone and joint pains were considered symptoms of yaws, because of the high percentage of reactors with this complaint. Both early and late bone lesions are included in this group of yaws lesions. Sometimes confusion arises because bone lesions can appear in children as early as the first year of infection. They may co-exist with other infections or with late lesions (multiple papillomata or hyperkeratosis). The early

bone lesions were often accompanied by inflammatory reaction of the bone, subjective pain, or painful tumefaction. Among the late bone manifestations most frequently seen were nodular tumefaction of the bone or deformation, sometimes in connection with ulcers of the skin, or with extensive or deforming scars.

9. *Latent cases.* It is routine to consider patients with a history of yaws or typical scars as latent cases. We can agree with this point of view when mass campaigns are in progress during which it is more desirable to make errors of diagnosis on the positive side than the reverse. However, in tropical Africa, experience has proved that a history of yaws is not of much value. People like injections and generally anybody wanting an injection during the surveys will claim a history of yaws. On the other hand some persons with typical lesions strongly deny having had yaws. Furthermore, a history of yaws in the past does not mean that, at the time of examination, a person has the disease. The differential diagnosis between typical scars of yaws and scars due to traumata or other causes is also very difficult, if not impossible. Africans have a lot of scars from many different causes. Serology is really the most valuable means of establishing latency with any certainty, but it is practically impossible to carry out systematic serology in mass field campaigns. On the other hand seropositivity may be caused by the existence of other treponematoses or other diseases. In Liberia, for instance, the prevalence of syphilis is not known and in villages on the main roads we have seen typical initial lesions of syphilis.

10. *Other manifestations.* In this group of lesions were chiefly included the dyschromias (leucoderma being more common), tenosynovitis or fascia lesions (sometimes associated with palmar hyperkeratosis), the juxta-articular nodules and ganglion of the wrist (sometimes with signs of fibrosis). These lesions were predominant in adults: in 763 cases observed, only 12 cases were recorded in the age group 2–18 years. It was remarkable that the number of cases seen in women was double that observed in males.

Dyschromia was the most frequent alteration observed, the leucoderma ("pintoid" yaws) being predominant mainly on hands and feet in the familiar "glove and stocking" pattern, sometimes associated with residual hyperkeratosis. The leucoderma frequently extended to the dorsum of the extremity. A great deal of tenosynovitis was seen associated with palmar hyperkeratosis and in late stages was accompanied by ankylosis and deformation of the joints. The small finger was most often affected and sometimes the lesions were bilateral.

Systemic symptoms of the disease were not completely investigated. No pure lesions of the mucosa were observed except some skin papillomata of the face near the borders of the mouth and nostrils which invaded a part of the corresponding mucous membranes. Sometimes yaws co-existed with iritis in children, but no evident relationship of cause and effect was proved. Association with leprosy was also observed.

Distribution of lesions by sex. The general distribution of yaws as regards sex was 3,105 cases in males (46.2%) and 4,787 in females (53.8%). However, this difference seems to be related to the fact that there was a higher attendance of women than men during the surveys. The incidence by sex according to the type

of lesion is shown in Table 3. At all ages the number of females with each type of lesion was greater than that of the males except in multiple papillomata, bone and joint lesions, and latent cases, which were more commonly seen in males. The most conspicuous differences in favor of females was observed in gangosa and the group of "other manifestations." Gangosa was only observed in females, and leucoderma and juxta-articular nodules were more often observed in females than in males.

Differences were observed between the sexes in the age group 2–10 years in multiple papillomata (3 males to 2 females) and hyperkeratosis (4 males to 3 females). Perhaps this indicates a relationship between hyperkeratosis and multiple papillomata: the former seems to be more prevalent when the latter is more frequent. This was true for wet crab yaws also; and here too, the number of males recorded was greater than the number of females.

Distribution of lesions by age. The overall incidence of yaws in relation to age is indicated in Table 3. The most frequent lesions observed in the different age groups were as follows. In infants (age group under 2 years), who were 0.7% of those examined: multiple papillomata, early skin lesions and bone and joint lesions; in children (age group 2–10 years), 21.9%: hyperkeratosis, multiple papillomata, wet crab, early skin lesions, gummata and ulcers and bone and joint lesions; in adolescents (age group 11–18 years), 12.9%: hyperkeratosis, early skin lesions, bone and joint lesions and gummata and ulcers; in adults (age group over 19 years), 64.5%: hyperkeratosis, other manifestations, bone and joint lesions and gummata and ulcers.

Follow-up surveys. The follow-up surveys were performed in some villages six months after the original surveys and treatment. Attendances were very poor and it was only possible to re-examine 40 per cent of the patients treated. Two dosage schedules were used (PAM in a single injection) as indicated in Table 4.

The results of the follow-up surveys are shown in Table 5. It should be added, however, that 681 contacts protected with the dosages indicated in Table 4 were re-examined and only one was found to have an initial lesion.

LABORATORY ASPECTS

Darkfield examinations. Seventy-seven darkfield examinations were made in patients attending the Kpain clinic, which also served as a treatment center for

TABLE 4

Treatment schedules used showing dosages in cc. (1 cc. PAM = 300,000 U)

	Age Groups	Under 2	2-10	11-18	Over 19
Schedule A	Infectious and late	1	2	3	4
	Latent cases	*	1	2	2
	Contacts	0.5	1	1.5	2
Schedule B	Infectious and late	2	3	4	6
	Latent cases	*	2	2	4
	Contacts	0.5	1	1.5	2

* Not treated.

TABLE 5

Clinical results following therapy with two different dosage schedules

Schedule	No. of Patients Re-examined	Percentage of			
		Cured	Improved	Same stage	Relapse
A*	300	56.3	29.3	13.7	0.7
B*	850	64.6	23.4	11.9	0.1

* As in Table 4.

TABLE 6

Results of darkfield examinations according to the type of lesion

Type of lesion*	No. Examined	Negative		Doubtful		Positive	
		No.	%	No.	%	No.	%
1	14	—	—	—	—	14	100.0
2	26	—	—	1	3.8	25	96.2
3	27	3	11.1	5	18.5	19	70.3
4	10	4	40.0	5	50.0	1	10.0
Total.....	77	7	9.1	11	14.3	59	76.6

* See Table 3.

yaws. Darkfields were performed on initial lesions, multiple papillomata, wet crab yaws and early skin lesions with the results indicated in Table 6.

It must be pointed out that some of the negative results in infectious cases were due to the application of local remedies, in the form of powder or ointment to fresh lesions.

Treponema were found in 14 initial lesions (100%), in 25 multiple papillomata (96.2%) and in 19 wet crab yaws (70.3%). The search for *Treponema* in the other early skin lesions was positive only in one case (10.0%). The scarcity of the positive results in this group of lesions is explained by the fact that many of these lesions are of allergic nature. Moreover, some clinical mistakes in diagnosis must be expected.

Serological data. The results of serology presented in this study give some information about the reactivity of the serum and the distribution of reagin titers according to the different types of lesion, the age and the sex of the patients. At the same time, as serology is the most reliable way to detect latent cases, surveys were made to determine the percentage of reactors among persons without clinical signs of yaws. Latent cases are of great epidemiological significance for the future of mass campaigns in Africa, and this subject held our special attention. Only results obtained with the VDRL tests are reported.

Qualitative tests. Among the population examined, 1,677 VDRL tests were performed without regard to the presence of clinical yaws; 1,177 (70.2%) persons were reactive and 500 (29.8%) non-reactive. This shows the high percentage of reactors in the African population examined and confirms the estimate that the

TABLE 7

Sero-reactors among persons with clinical signs of yaws and persons without signs of yaws (regardless of history)

	No. Tested	No. Non-reactive	Reactive	
			No.	%
Cases with clinical yaws.....	920	96	824	89.6
Persons without signs of yaws....	757	404	353	46.6
Total.....	1,677	500	1,177	70.2

TABLE 8

Sero-reactors among yaws patients by type of lesion

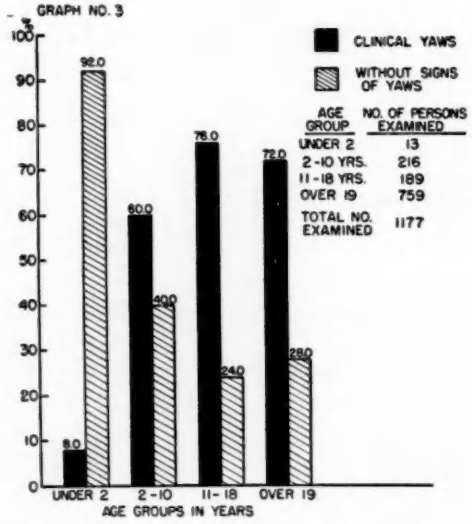
Type of Lesion*	No. Tested	No. of Non-reactives	Reactives	
			No.	%
1	6	1	5	83.3
2	28	4	24	85.7
3	17	0	17	100.0
4	16	4	12	75.0
5	649	72	577	88.9
6a 6b	17	4	13	76.5
7	42	2	40	95.2
8	52	3	49	94.2
9	93	6	87	93.5
Total	920	96	824	89.6

* See Table 3, footnote.

real prevalence of yaws is around 70 per cent. The relative percentage of reactors between yaws patients (clinical yaws) and persons clinically negative for yaws is indicated in Table 7. Almost 47 per cent of persons without clinical signs of yaws were reactive, and these, excluding those reacting to other causes present in Africa of non-treponemal etiology (rare in our opinion), are presumably latent cases of yaws. This shows that in mass campaigns in Africa, latent cases should be carefully considered from the epidemiological standpoint.

Positive qualitative tests according to age. Of the 1,177 persons examined serologically and found positive, including clinical and nonclinical yaws, 1.1 per cent were under 2 years of age; 18.4 per cent, 2–10; 16.1 per cent, 11–18; 64.4 per cent, over 19 years old. Of these reactors, 824 were clinical cases and 353 were persons without signs of yaws (Tables 7 and 8). The percentage of each age group clinically positive or negative is shown in Graph 3.

In patients with yaws, it is seen that the percentage of reactors increased rapidly in the early years and reached its maximum in adolescence, after which there was no significant change, but the percentage of reactors among persons without signs of yaws was highest in the age group under 2 (92.3%) and decreased with age. This may be due to the presence of reagins passed from mother to



GRAPH No. 3. Relative percentages of reactors with and without signs of yaws, by age groups.

child; quantitative serological tests in babies would be necessary to show whether a decrease in the titers of reagin would give support to this assumption.

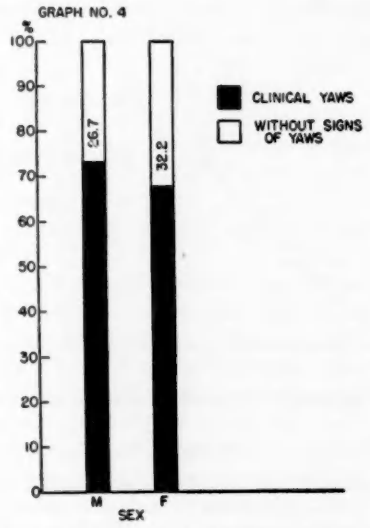
Positive qualitative tests by sex. The percentages of clinical and non-clinical cases of yaws of each sex, among 1,177 persons tested serologically and found positive, are shown in Graph 4. Females outnumbered males in both groups: of 824 reactors with clinical yaws, 476 were female and 348 male; of 353 reactors without signs of yaws, 226 were female and 127 male.

Something over 10 per cent were non-reactive. The minimum percentage of reactors was observed in early skin lesions (75.0%) and the maximum (100.0%) in wet crab yaws. The group of lesions in which the possibility of clinical errors is greatest (other early skin lesions and ulcers) gave proportionately the highest percentage of negative sero-reactors.

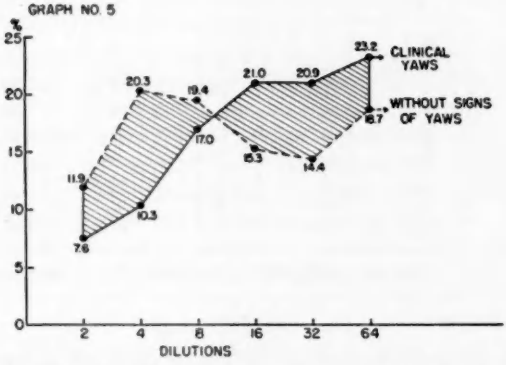
Quantitative serological tests. It was possible to make quantitative VDRL tests in 1,057 sera: 737 in patients with clinical yaws and 320 in persons without clinical signs of yaws. Dilutions were made only to 1:64 because the serological experience has shown that the majority of the sera in yaws are reactive up to 64 dilutions. The results are indicated in Graph 5.

In the curve of the yaws patients, the per cent of individuals with each titer increased regularly with the dilution, reaching the maximum at the highest titer, 1:64. In the curve of persons without signs of yaws, the peak was reached at the 1:4 dilution and then decreased, showing many fewer cases with titers between 16 and 64 than the yaws patients.

Quantitative tests according to age. Quantitative tests were performed in 737

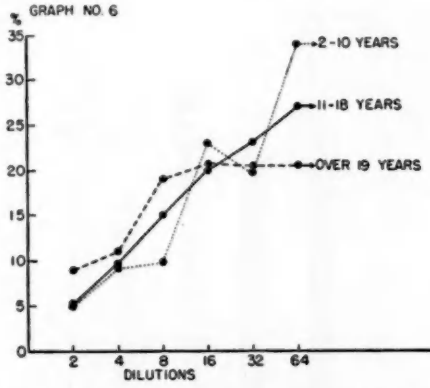


GRAPH No. 4. Relative percentages of 1177 reactors with and without signs of yaws according to sex.



GRAPH No. 5. Distribution of 1057 reactors with and without signs of yaws according to reagin titers.

yaws patients, comprising 1 case under 2 years of age, 116 cases between 2–10 years, 138 cases between 11–18 years and 142 cases over 19 years. The results in detail are indicated in Graph 6. Regardless of the type of lesion, it may be said that at all age groups the titers of the greatest number of reactors fell between 16 and 64. The curve of the age group 2–10 years runs the gamut from the minimum to the maximum. The curves of both children and adolescents show a regular increase in reagin titers and the curve of adults has the same pattern up to 8 dilutions, showing a great stability at higher titers.



GRAPH No. 6. Comparison of titers of 737 reactors according to age group

TABLE 9

Quantitative serological testing (VDRL) in yaws patients by type of lesion

Type of Lesion*	No. Reacting	Dilutions											
		1,2		1,4		1,8		1,16		1,32		1,64	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	5	1	20.0	—	—	—	—	—	—	1	20.0	3	60.0
2	17	1	5.9	3	17.6	4	23.5	3	17.6	3	17.6	3	17.6
3	17	—	—	3	17.6	1	5.9	3	17.6	4	23.5	6	35.3
4	12	1	8.3	2	16.6	—	—	2	16.6	2	16.6	5	41.7
5	523	42	8.0	52	9.9	90	17.2	177	22.4	108	20.7	114	21.8
6a 6b	9	1	11.1	—	—	2	22.2	—	—	2	22.2	4	44.4
7	33	4	12.1	4	12.1	6	18.2	5	15.2	3	9.1	11	33.3
8	44	4	9.1	2	4.5	13	29.5	7	16.1	12	27.2	16	13.6
9	77	2	2.6	10	12.9	9	11.7	18	23.4	19	24.7	19	24.7
Total	737	56	7.6	76	10.3	125	17.0	155	21.0	154	20.9	171	23.2

* See Table 3, footnote.

Quantitative serological tests according to the type of lesion. The distribution of reagin titers by type of lesion in 737 VDRL quantitative tests is indicated in Table 9. The greatest percentage of reactors fall in the dilutions between 16 and 64. The number of reactors in each dilution increases regularly from the lower dilutions to the higher.

1. *Initial lesion.* In five quantitative tests, the largest percentage of reactors fell in the 64-dilution group. One case was non-reactive and another reactive only at the 2-dilution level. It is known that the reagin appears in the blood during the 6th week after infection and for this reason it is possible that some early initial lesions would be non-reactive because there had not been sufficient time to produce a detectable increase of reagin.

2. *Multiple papillomata.* The distribution of cases at different dilutions in 17 quantitative tests is uniform and no differences were observed. It shows that the reagin titer in this type of lesion has a certain degree of stability. The maximum number of cases appear at the 8-dilution level.

3. *Wet crab yaws.* It may be said that wet crab yaws gave the highest degree of positivity. Most cases went beyond the 1:16 dilution, reaching the maximum at 64 dilutions. The pattern of the distribution of the cases is a bit different from that of multiple papillomata.

4. *Other early skin lesions.* The greatest number of cases in 12 quantitative tests were at the 1:64 level.

5. *Hyperkeratosis.* In 523 quantitative tests the number of reactors increased regularly from the lower dilutions up to the highest, and the majority of the cases fell between 16 and 64 dilutions. This agrees with the clinical findings, which showed a large number of active plantar and palmar hyperkeratoses. The non-reactive cases include naturally some regressive or residual hyperkeratosis, many of which would also be included among the reactors at lower dilutions. Some others are the result of diagnostic errors as previously noted.

6. *Gummata, ulcers and gangosa.* Nine quantitative tests were performed. The highest percentage of reactors with gummata, ulcers and gangosa were at the 64 dilutions level. Among the non-reactive cases must be included some ulcers of non-treponemal etiology.

7. *Bone and joint lesions.* The 33 quantitative tests performed show that about one third of cases were at the 64 dilutions level.

8. *Latent cases.* Forty-four quantitative tests show that the highest percentage of reactors is at the 16-dilution level. This suggests a stationary or regressive type of yaws.

9. *Other manifestations.* In seventy-seven quantitative tests the reactors showing the highest titers were at the 32–64 dilutions level. The general pattern of the distribution of these reactors has the same aspect as that of the hyperkeratoses, increasing from lower dilutions up to the highest.

Because of the small numbers of reactors in some of the groups of lesions examined it is not possible to make more detailed comparisons.

SUMMARY

As yaws is one of the most important problems of the public health program in Liberia, clinical and serological studies were made during a combined malaria and yaws pilot project as a preliminary to a mass campaign. In the 8,892 cases of yaws examined (46.2 per cent males, 53.8 per cent females) hyperkeratosis was the most frequent lesion in those over two years old, followed by infectious lesions of the 2–10 year olds and the group of miscellaneous manifestations in those over 19. In 77 darkfield examinations of infectious cases, the initial lesion was 100 per cent positive, while multiple papillomata was positive in 96.2 per cent and wet crab yaws in 70.3 per cent. The VDRL serological test was positive in 75 per cent of patients with early skin lesions and in all those having wet crab yaws; in 757 patients without clinical signs of yaws, 46.6 per cent were positive.

A follow-up survey of 1,000 individuals six months after treatment with penicillin (PAM in a single injection) showed about 65 per cent cured, 25 per cent improved, and the rest unchanged. Only 0.1 per cent had relapsed.

ACKNOWLEDGMENT

We would like to express our thanks to the Government of Liberia for permission to publish this paper.

THE LABORATORY DIAGNOSIS OF TYPHOID FEVER IN AREAS OF ENDEMICITY

K. C. WATSON AND W. LAURIE

Edendale Hospital, Pietermaritzburg, Natal, South Africa

Typhoid fever still remains an endemic disease in various parts of the World, particularly in tropical and subtropical regions. Diagnosis of the classical case presents little difficulty but in many instances the clinical picture is atypical, and laboratory tests are essential in making the diagnosis. Such tests fall into two main groups, viz., isolation of the causative organism and serological procedures. This paper deals with our findings in a group of two hundred and thirty nine suspected cases of typhoid fever and is an attempt to assess the relative advantages and disadvantages of these two diagnostic procedures. All the cases were sporadic ones.

MATERIALS AND METHODS

Whole blood cultures. These were performed by the addition of eight to ten ml. of blood to fifty ml. of 0.5% bile salt broth in sterile eight-ounce bottles. The bottles were incubated at 37.0°C. and subcultures were plated daily on to Wilson and Blair's bismuth sulphite medium. All culture bottles were kept for three weeks before being discarded.

Culture of Blood Clot. The serum from a five ml. volume of clotted blood was removed for estimation of agglutinin levels. The residual clot was then added to fifteen ml. of 0.5% bile salt broth containing, one hundred units per ml. of streptokinase, in a universal container. All containers were then incubated at 37.0°C. and subcultures plated daily as for whole blood cultures. The streptokinase causes rapid lysis of the clot with release of trapped organisms into the medium (Thomas *et al.*, 1954). Using this method we found that culture bottles could be discarded after one week since positive cultures were almost always detected during the first forty-eight hours of incubation. Later in the investigation a modification of this method was employed, the streptokinase bile broth and the Wilson and Blair agar slope being combined in an eight-ounce bottle (Watson, 1956). This modification reduced the time necessary for a positive isolation even further.

Agglutinin titers. The antigen suspensions used by us were prepared from the standard O 901 strain of *S. typhi* (obtained from Colindale Laboratories, London). The tests were carried out in 3" by ½" tubes over a serum dilution range of 1/125 to 1/1,000. These tubes were found to be more satisfactory than the standard Dreyer tubes. The tubes were incubated for twelve hours in a 52.0°C. water bath before being read. Only O type granular agglutination was tested for. We discuss later our reasons for discarding H type agglutination.

Stool and Urine Cultures. Stools were plated on desoxycholate citrate agar, Wilson and Blair agar, and into selenite F medium. Subcultures from the selenite

were plated on desoxycholate citrate agar six hours after incubation. Suspicious colonies were picked off and checked in the usual way for biochemical and serological reactions.

Urine deposits were plated on to Wilson and Blair's agar and MaConkey agar.

RESULTS

Table 1 shows the comparative results of isolation of *S. typhi* (from clot and blood cultures) and the corresponding serological reactions in 239 patients suspected of having typhoid fever. The term "positive Widal" has been taken as meaning a minimum agglutinin titer of 1/250. Reasons for accepting this figure are discussed below.

On further investigation of group 3 (negative isolation and positive Widal reaction) it was found that twelve of the thirty-eight patients were not suffering from typhoid fever in spite of their showing O agglutinin levels of 1/250 or greater. This group of twelve false positives presented a variety of illnesses with pyrexia, one being an infection with *S. typhimurium*. The remaining twenty-six patients appeared to be genuine cases of typhoid fever. Eight of them had positive stool cultures and the remainder presented typical clinical features of the illness.

In each patient the samples for both types of blood culture were collected simultaneously.

From group 2 in Table 2 above it will be seen that in seventeen patients clot culture was negative where the whole blood culture was positive. This is explained by the small volume of clot submitted for culture in these cases, often not more than half a ml. Such small clots were probably sterile since in the

TABLE 1

Cultural and serological findings in 239 pyrexial patients

Group	Blood Cultures	Widal Results	No. of Patients	%
1	Positive	Positive	133	55.6
2	Positive	Negative	68	28.4
3	Negative	Positive	38	16.0
Total.....			239	100

TABLE 2

Results of blood cultures

Group	Blood Culture	Clot Culture	No. of Patients	%
1	Positive	Positive	96	43.0
2	Positive	Negative	17	7.6
3	Negative	Positive	69	30.9
4	Not done	Positive	19	8.5

i.e Total number of positive blood cultures = 113 (62.0%). Total number of positive clot cultures = 184 (82.4%).

average patient with bacteremia the number of organisms per ml. of blood is very small.

In all, *S. typhi* was recovered from the blood stream of 201 patients in this series of 227 proved cases of typhoid fever. This represents a recovery rate of 89 per cent, a figure which compares favorably with the findings of other workers. Chatterjee (1942) recorded a maximum isolation rate of 75 per cent during the second week of illness in a series of 348 cases. Stuart and Pullen (1946) had 81 per cent positive isolations during the patients' first week of illness, and Shaw and MacKay (1951) obtained 90 per cent positive isolations in the first week of illness and 84 per cent in the second week. Incidentally we have confirmed the view of Shaw and MacKay that positive isolations may be obtained at any stage of the illness and not, as is often taught, only during the first week.

DISCUSSION

Cultural findings. The cultural results detailed above clearly indicate the importance of seeking the causative organism as being the main diagnostic procedure in typhoid fever. In spite of the fact that many of our patients had been treated with antibiotics before blood was taken for culture, and in spite of the small volume of blood often submitted, our isolation rate has proved most satisfactory.

The findings in Table 2 show the superiority of the technique of culture of blood clot compared with whole blood culture, there being 82.4 per cent positive isolations from the former compared with 62 per cent from the latter. We have previously discussed some of the reasons for this (Watson, 1955). One main reason for this difference is that, unless very large volumes of culture medium are used to dilute out bactericidal serum factors which may affect the organisms, whole blood culture methods will not be as satisfactory as they should be. This is especially the case where the degree of bacteremia may be very low.

With the clot culture technique it is essential that some lytic agent be incorporated in the medium to allow of rapid disintegration of the clot and consequent release of the trapped organisms. For this purpose we have found streptokinase to be more satisfactory than agents such as trypsin. Streptokinase in the concentration used, 100 units/ml., has no effect on the viability of the organism.

Diagnosis of the relapse phase of typhoid fever is usually obvious from the clinical features and in our experience isolation of the causative organism from the blood stream can be made just as readily as in the initial attack. The administration of chloramphenicol during the first attack does not appear to affect such isolation in the relapse state although other workers have claimed that it does (Marmion, 1952).

Stool and urine cultures. Isolation of *S. typhi* from stools and urine has proved disappointing in our experience. Recovery was made on 64 occasions from the former but only on three from the latter. It may be that the administration of chloramphenicol prevents the development of a renal lesion which may be necessary for the discharge of organisms into the urine. Neither of these methods however, is of practical importance in the first week of illness.

Serological findings. In view of the fact that typhoid fever is endemic in this

area it is probable that we were dealing with a semi-immune population in our series. Consequently, we have not recorded the H agglutinin levels although by doing so we may have missed that small proportion of typhoid fever patients who only develop H agglutinins.

In this area there are other factors which complicate the interpretation of serological findings. Thus, the patient comes to hospital late in the course of illness; previously he may have had contact with *S. typhi* or other organism of related antigenic structure; he may have been inoculated with T.A.B. vaccine; the population of the area may show relatively high levels of naturally occurring agglutinins; and the patient often refuses to allow the taking of more than one sample of blood.

The laboratory cannot refuse to investigate the titer levels of single specimens of blood although it is generally accepted that it is unwise to select any arbitrary titer as being "positive". One object of this paper is to assess the value of titer levels in such single specimens. Lewin (1938) and Topley and Wilson (1948) consider that it is essential to establish first the minimum diagnostic titer in any particular area. Lewin suggested that (1) the titer levels should be of such value as to diagnose the large majority of typhoid cases, i.e., they should not be so high as to cause false negative results to be returned; (2) they should be low enough to diagnose the disease in its early stages; (3) they should not be so low as to include cases whose serum contains normal agglutinins.

From a survey of the titer levels in 442 individuals with no history of T.A.B. vaccination and no history of having had typhoid fever, Lewin suggested that diagnostic titers in South Africa should be 1/100 for H agglutination and 1/200 for O agglutination. Alves (1936) in Southern Rhodesia concluded that an O agglutination level of 1/250 was diagnostic. In other parts of the world various other levels have been suggested. Horgan (1932) took 1/50 as the upper level of "normal" agglutinins in the Sudan. Giglioli (1933) found that only 0.3 per cent of healthy workers in British Guiana gave O titers of 1/160 or above. Soman (1934) working with an Indian civil population, took 1/50 or above as being significant. Stuard and Pullen (1946) based their opinion on the titer levels in 360 patients with typhoid fever and concluded that 1/160 was the minimum diagnostic O titer. It should be noted that one-third of their proved cases failed to reach this level. Topley and Wilson (1948) reviewed the literature and concluded that in England, the diagnostic titer should be H 1/50 and O 1/100. This agrees with the observations of Schwabacher *et al.* (1943) who investigated 560 normal sera in England and found that, with practically no exceptions, the highest O titer was 1/80.

The above authors all base their minimal diagnostic titers on the assumption that the patients have not been vaccinated with T.A.B. and have not previously suffered from infection with *S. typhi*, or an organism of related antigenic structure. In areas where typhoid fever is endemic it is safer to base minimal diagnostic titer levels on the assumption that one is dealing with a semi-immune population. This would explain the high levels suggested by Lewin and Alves compared say, with those suggested by Schwabacher *et al.* Grasset (1945) men-

tions the limitations of use and interpretation of serological tests in uninoculated groups.

In areas where the population is semi-immune it is difficult to suggest a minimum diagnostic titer. During and after the first world war the general opinion was that the Widal reaction was of little diagnostic value in individuals who had been inoculated. Felix's (1924) theory of "qualitative receptor analysis" was soon disproved and the pessimistic view of the usefulness of the Widal received strong support, e.g., Lewin (1938), Horgan (1932) and Grasset (1945). Grasset based his views on the findings in 183,000 inoculated healthy mine-workers.

Other workers have reported contrary views. Gardner (1929) considered that an O level of about 1/200 was probably diagnostic of infection regardless of vaccination. Bole (1935) from her experience of testing single samples of blood from pyrexial patients, suggested that a titer of 1/320 was sufficiently high to exclude non-specific O agglutination. Anderson and Richards (1948) reported on titer levels in 110 R.A.F. personnel with typhoid fever. (Over 90 per cent of their patients had been inoculated with T.A.B. within the previous year;—Group Captain J. S. Wilson, personal communication). Even under the stimulus of active infection the titer levels were found to be;

O titer of 1/200 or above (third week of disease) = 65 % of serious cases
67 % of moderate cases
50 % of mild cases.

Downie (1942) investigated the titer levels in healthy inoculated R.A.F. personnel; his findings are tabulated below.

Time Since Last T.A.B. Injection	No. of Sera Tested	Suspension	Percentage Sera with End Titers of					
			Under 1/100	1/100–1/200	1/200–1/400	1/400–1/800	1/800–1/1600	1/1600 and over
1 week to 3 months	198	TO	95	5.0	—	—	—	—
		TH	30.3	21.7	23.8	16.1	6.6	1.5
		AH	5.0	7.6	15.7	30.3	29.8	11.6
		BH	4.0	10.1	20.2	24.8	27.2	13.6
3 months to 1 year	70	TO	97.1	2.9	2	—	—	—
		TH	37.2	25.7	20.0	12.9	4.3	—
		AH	15.7	21.4	12.9	30.0	14.3	5.7
		BH	10.0	10.0	21.4	28.6	18.6	11.4
1 year to 3 years	29	TO	100.0	—	—	—	—	—
		TH	41.4	31.0	13.8	3.8	10.3	—
		AH	34.5	—	20.6	20.6	10.3	13.8
		BH	17.2	20.6	13.8	31.0	10.3	6.9
Total sera 1 week to 3 years	297	TO	96.0	4.0	—	—	—	—
		TH	33.0	23.6	21.9	14.1	6.4	1.0
		AH	10.4	10.1	15.5	29.2	24.2	10.4
		BH	6.7	11.1	19.9	26.2	23.6	12.5

Downie found that even in individuals inoculated repeatedly over a three-year period the resultant O titers were low in level and were transitory. He further

found that anamnestic reactions were uncommon. Downie concluded that in inoculated persons estimations of O agglutinins are of considerable diagnostic value, with titers of 1/100 suspicious, and rising titers and those above 1/200 strongly suggestive of typhoid fever. Topley and Wilson (1948) agree with these observations.

In dealing with semi-immune populations such as we have had experience with here, we consider that the minimal suggestive titer is 1/250, a figure close to that suggested by Lewin (1938) and Alves (1936) for uninoculated individuals. It is agreed that O type agglutination only identifies enteric group infection, but in this area paratyphoid B does not occur and paratyphoid A is very uncommon.

Accepting a minimum suggestive titer of 1/250 we found 28% of our proved typhoid patients (blood culture positive) to be serologically negative. This was also the finding of Stuart and Pullen (1946) and Anderson and Richards (1948). It would seem therefore that the Widal reaction will fail to detect almost one-third of true typhoid fever cases. With this titer level of 1/250 we obtained agreement between culture findings and the Widal reaction in 56 per cent of our series. In 11% of the cases the serological findings were positive where the blood culture was negative, the latter finding being presumably due to previous antibiotic treatment or too small samples of blood. In the remaining 5% of cases the Widal test gave false positive reactions, presumably due to anamnestic rises in the titers except in one patient suffering from infection with *S. typhimurium*. This organism has a common X11 antigen with *S. typhi*.

SUMMARY

A study of cultural and serological methods of diagnosis in a series of 227 cases of typhoid fever demonstrated that the former methods were to be preferred. In particular culture of blood clot is a more satisfactory procedure than culture of whole blood. Our total recovery rate from blood culture methods was 89%, and this in spite of many patients having received antibiotic therapy before blood samples were taken. Accepting a serum dilution of 1/250, for O type agglutination, as being the minimal suggestive titer we found 5% false positive reactions. Twenty eight per cent negative results were obtained. Reasons are discussed for accepting the figure of 1/250 minimal dilution of serum as being suggestive of infection in this area.

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STUDIES ON *PASTEURELLA PESTIS* IN FLEAS. V. THE EXPERIMENTAL PLAGUE-VECTOR EFFICIENCY OF WILD RODENT FLEAS COMPARED WITH *XENOPSYLLA CHEOPIS*, TOGETHER WITH OBSERVATIONS ON THE INFLUENCE OF TEMPERATURE

LEO KARTMAN AND FRANK M. PRINCE

San Francisco Field Station, San Francisco, California[1]

It is now 48 years since McCoy (1908) and Wherry (1908) first demonstrated plague in ground squirrels, *Citellus beecheyi beecheyi*, in California. Since that time, plague surveys by the U. S. Public Health Service and certain states have shown the infection to be present in wild rodents and their fleas from fifteen states west of the 100th meridian (Wayson, 1947; Link, 1950). It is an established fact that a large wild-rodent reservoir of plague exists independent of domestic rats and, in the words of Meyer (1942), ". . . thus making the situation potentially more dangerous than in the past, and further adding complications to a situation which already in 1908 appeared formidable in all its ramifications."

The plague vector capacity of wild rodent flea species became a matter for careful consideration in studies oriented toward explaining the ecology of wild rodent plague. Within the last two decades various fleas have been subjected to laboratory tests of their vector ability and many species have been compared with *Xenopsylla cheopis* in this respect (Eskey, 1938a; Eskey and Haas, 1940; Wheeler and Douglas, 1941; Wheeler and Douglas, 1945; Burroughs, 1947; Holdenried, 1952; Kartman *et al.*, 1956). These studies have indicated the experimental potentials for plague transmission of a number of wild rodent flea species. When such information is correlated with ecological and epizootiological information, a much more complete picture is obtained of the potential role of a flea species in relation to wild rodent plague.

Such studies have been made even more imperative in recent years since it is suspected that the smaller wild rodents such as *Microtus* spp. and *Peromyscus* spp. may be the more usual plague reservoirs rather than the squirrels and other larger rodents (Meyer, 1955). The dangers inherent in the close association of wild rodents and domestic rats was recognized over forty years ago by McCoy (1910), and, more recently Stewart (1940) paid particular attention to the danger of transfer of fleas between wild and domestic rodents. On the basis of evidence that *Malaraeus telchinum* has been found both on domestic rats and wild rodents (Eskey, 1938b), Stewart postulated that this species ". . . must be looked upon with suspicion." This hypothesis has been amply supported by the experiments of Burroughs (1944 and 1947) and by studies on the ecology of plague in native voles and field mice (Miles *et al.*, unpublished).

The present communication is a report of vector efficiency tests of various wild rodent fleas, many of which have not heretofore been recorded. Tests of

[1] From the Communicable Disease Center, Public Health Service U. S. Department of Health, Education, and Welfare, Atlanta, Georgia.

vector capacity of fleas held at different temperatures are also included. The experiments were conducted under the supervision of Dr. C. R. Eskey during the years 1947–1950, with the major share of responsibility assumed by one of the authors (FMP). It should be noted that, for reasons explained below, very few specimens of certain flea species were available, thus some of the results presented are primarily suggestive and need further confirmation.

MATERIALS AND METHODS

There is little need to describe the methods in detail since accounts already have been published (Link and Prince, 1950; Kartman *et al.*, 1956). Specific note should be taken of the fact that plague infection in the flea is here based upon confirmed culture of *Pasteurella pestis* from the flea after its death or sacrifice. In all cases, individual fleas were fed upon single mice. The mice were then held for at least 10 days and, if they had not succumbed to confirmed plague infection, were then killed and tested bacteriologically for the presence of plague organisms. Although, in the majority of cases, the plague transmissions represent the results of infective flea feedings on successive days, in one instance feedings by an infective flea upon more than one mouse in a single day were accomplished. This case is noted in the data (Table 4). It should be mentioned that the level of plague transmission from single flea feedings followed by removal of the flea, although useful in deriving comparative vector efficiency indices, is probably below that which occurs with continuous and/or mass exposure in nature.

In the majority of instances, the infected fleas were maintained at room temperature. Daily records of temperature fluctuation were obtained and the average temperatures are shown in the tabular data. In some experiments the fleas were kept in a refrigerator which fluctuated between 6°–10°C., and in other tests the fleas were held in an incubator at between 28°–32°C. Open pans of water were placed in the incubator to supply moisture, but under the other conditions, mentioned above, no source of moisture in addition to that already in the atmosphere was supplied.

The fleas used in these experiments were either reared in the laboratory, shipped from the field, or brought from the field by survey crews. Many fleas were taken from rodents in the field, but quite often they were taken with the host's nest which was placed in a thermos jug for transport or shipment to the laboratory. In many instances fleas in nests were collected at great distances from the laboratory and the supply of fleas that was eventually available for the transmission experiments varied considerably. Since no suitable culture techniques have been developed for rearing many wild rodent fleas, the experimental work depended upon the number of adult fleas present in the collected material and upon adults which could be reared from eggs, larvae, or pupae also present in the nests. All fleas used were specifically identified.

The species of fleas and their collection records from 4 different localities are as follows: (1) from the San Francisco bay area; *Xenopsylla cheopis* from domestic rats and reared in the laboratory; *Diamanus montanus* from *Citellus* sp. and laboratory bred; *Atyphloceras multidentatus* from nests of *Microtus californicus*; *Hystrichopsylla* sp. (possibly a subspecies of *occidentalis*; Miles *et al.*,

unpublished) from *M. californicus* nests; *Leptopsylla segnis* from *Mus musculus*; (2) from Kittitas County, Washington; *Catallagia decipiens* from *Lagurus curtatus*; *Monopsyllus wagneri wagneri* from *Peromyscus maniculatus*; *Thrassis bacchi johnsoni* from *L. curtatus*; *Megabothris clantoni johnsoni* from *L. curtatus*; *Meringis shannoni* from *Perognathus parvus*; (3) from Nebraska; *Monopsyllus exilis kansensis* from *Onychomys leucogaster*; *Thrassis fotus* from *O. leucogaster*; *Epitedia wenmanni wenmanni* × *testor* from *P. maniculatus*; (4) and *Megabothris abantis* from *Microtus montanus* in Teton County, Wyoming.

The derivation of numerical values for vector efficiency follows the method described by Wheeler and Douglas (1941, 1945) and the modifications of this method suggested by Kartman *et al.* (1956). The vector index proposed by Kartman *et al.* (1956) is also used. This is the product of the vector efficiency and the blocking-survival potential (mean day of death after blocking/mean day of blocking after infectious meal).

DATA AND DISCUSSION

Diamanus montanus

Successful mass plague transmission experiments with *D. montanus* were carried out over 45 years ago by McCoy (1911). Eskey (1938a) and Eskey and Haas (1940) were the first to test the plague vector capacity of individual fleas of this species.

Table 1 presents a summary of the results with this species and the data of other workers for comparison. The data have been arranged in conformity with

TABLE 1

The efficiency of Diamanus montanus *as a vector of plague to albino laboratory mice*

Approx. Mean Temp. (°C)	No. Used	No. Infect.	No. Blocked	No. Trans.	Infect. Poten.	Block. Poten.	Trans. Poten.	Vector Efficiency	Source*
8	40	15	0	0	0.37	0	0	0	Present report (1948)
19	72	19	2	3	0.26	0.10	1.50	0.04	Eskey and Haas (1940)†
21	183	65	9	8	0.35	0.13	0.88	0.04	Present report (1948)
21	66	17	2	1	0.25	0.11	0.50	0.02	Burroughs (1947)
21	48	41	21	50	0.85	0.52	2.38	1.04	Wheeler and Douglas (1941)
21	80	67	32	67	0.84	0.48	2.09	0.84	Wheeler and Douglas (1945)
21	51	39	20	42	0.76	0.51	2.10	0.82	Wheeler and Douglas (1945)

* Where authors' names are shown, the date is the year of publication; other dates are the years in which the work was done.

† This work was done with guinea pigs and is thus not strictly comparable to the other work cited.

the temperature under which the infected fleas were maintained. Where the values for the various potentials were not originally presented (Eskey and Haas, 1940 and Burroughs, 1947), these have been calculated from the published data.

The vector efficiency data, presented here, fall into the following three groups: (1) those fleas held at a low temperature which were unable to become infective even though their infection potential was equal to that in several other groups of fleas held at higher temperatures; (2) those fleas with strikingly uniform potentials which had consistently low vector efficiencies; and (3) those fleas with consistently high vector efficiencies which also showed very uniform potentials.

There was an adverse effect of low temperature upon the ability of *P. pestis* to cause complete proventricular blockage in *D. montanus*. Whether or not this is the case with other flea species and with different strains within a species is not clear (see below). The other two groups of fleas have vector efficiencies which are statistically distinct. Burroughs (1947) has examined this problem and he concluded that the differences were due to a biological variation or strain difference in the fleas used. Holdenried (1952) also studied this problem by testing the vector capacity of *D. montanus* from six separate areas in California. He obtained no transmission by fleas from four of the areas and only poor transmission by fleas from two of the collection sites. Although his data do not allow calculation of the various potentials, a rough estimate from his figures shows an approximate vector efficiency of 0.02 which is the same as that obtained by Burroughs (1947). Eskey (1938a) also obtained very poor transmission with this species.

Calculation of the blocking-survival potentials and the vector indices corroborates the vector efficiency values of the strains showing poor and good vector capacities. The blocking-survival potentials and the vector indices were, respectively, as follows: present report, 0.18, 0.007; Burroughs (1947), 0.12, 0.002; and Wheeler and Douglas (1945), 0.50, 0.42. Here again there is a striking difference between strains which fall distinctly into two categories. The unusually high blocking-survival potential of the strain used by Wheeler and Douglas is based upon the remarkable survival of the infective fleas which ranged from 1 day to 51 days with an average of 5 days.

Recent studies by Evans *et al.* (1943) and Meyer and Holdenried (1949) have shown the importance of this species in wild rodent plague. The latter study has added concrete evidence to the hypothesis that these fleas may transfer plague from wild rodents to domestic rats, a view held by Prince (1943b) who recorded *D. montanus* from rats in three localities in California, three places in Oregon, and in one locality in Texas.

Xenopsylla cheopis

Although a summary of vector capacity experiments with *X. cheopis*, and other species of fleas, shows *X. cheopis* to be the plague vector par excellence (Pollitzer, 1954), its vector efficiency may vary due to a number of influences. Apparently, climatic factors are of critical importance to vector capacity, and

TABLE 2

The efficiency of Xenopsylla cheopis *as a vector of plague to albino laboratory mice in relation to temperature*

Approx. Mean Temp. (°C)	No. Used	No. Infect.	No. Block.	No. Trans.	Infect. Poten.	Block. Poten.	Trans. Poten.	Vector Efficiency	Source*
8	26	21	14	22	0.80	0.66	1.57	0.81	Present report (1948)
20	24	16	16	28	0.66	1.00	1.75	1.15	Present report (1948)
21	102	79	56	50	0.77	0.70	0.89	0.48	Present report (1948)
21	50	49	14	20	0.98	0.29	1.44	0.39	Wheeler and Douglas (1941)
21	49	47	14	21	0.96	0.30	1.50	0.43	Wheeler and Douglas (1945)
21	53	38	31	35	0.72	0.82	1.12	0.66	Burroughs (1947)
22	30	29	23	21	0.96	0.79	0.91	0.69	Kartman et al (1956)
30	76	65	25	14	0.85	0.38	0.56	0.12	Present report (1950)

* Where authors' names are shown, the date is the year of publication; other dates are the years in which the work was done.

consequently to the course of epizootics. Pollitzer has written an exhaustive summary of the literature on this subject. His review indicates that various workers have shown experimental transmission of plague to be two to three times as successful at temperatures of from 20°–24°C. than at 29°–32°C. Furthermore, there is evidence that plague epidemics are checked when the mean daily temperature rises above 27°C.

Tables 2 and 3 summarize data on the vector efficiency, extrinsic incubation period, survival after blocking, and the vector indices of *X. cheopis* kept at different temperatures during experiments to determine vector capacity. In general, it is apparent that the values for vector efficiency are higher at the lower temperatures. The surprisingly high value at a temperature of 8°C. is difficult to explain. At this thermal level, the vector capacity of *D. montanus* was nullified (Table 1), but the strain used was a very poor plague vector when compared to *X. cheopis* under more optimal temperatures (Tables 4 and 5). The vector efficiency of *X. cheopis* at a temperature of 30°C. was the lowest of all the groups shown. Holdenried (1952) kept infected *X. cheopis* at a mean temperature of about 21°C. and a rough estimate of vector efficiency from his published figures gives a value of 0.85. On the other hand, Eskey and Haas (1940) kept their fleas at a mean temperature of about 19°C. and, using guinea pigs, obtained plague transmissions which give a vector efficiency value of 0.19. However, the difference in techniques and experimental animals may account for these contradictory values.

TABLE 3

Extrinsic incubation, survival, and vector indices of plague-infected Xenopsylla cheopis *kept at various temperatures*

Approx. Mean Temp. (°C)	Extrinsic Incubation (days)		Death After Blocking (days)		Blocking-Survival Potential*	Vector Index†	Source‡
	Range	Mean	Range	Mean			
8	21–60	34.4	1–6	4.4	0.12	0.09	Present report (1948)
20	12–32	18.9	1–7	3.0	0.15	0.17	Present report (1948)
21	11–49	22.0	1–11	3.0	0.13	0.06	Present report (1948)
21	6–33	16.0	1–18	4.0	0.25	0.11	Wheeler and Douglas (1945)
21	5–25	12.6	1–17	4.4	0.34	0.22	Burroughs (1947)
22	13–42	23.5	1–8	3.7	0.15	0.10	Kartman et al (1956)
30	3–25	11.9	1–4	1.2	0.10	0.01	Present report (1950)

* Mean day of death after blocking/mean day of blocking after infectious meal.

† Vector efficiency × blocking-survival potential.

‡ Where authors' names are shown, the date is the year of publication; other dates are the years in which the work was done.

Some workers have assumed that the main effect of high temperature is to hasten the clearing by the flea of its ingested plague bacilli (Pollitzer, 1954). Others, like Eskey and Haas (1940), stress the effect upon the period of extrinsic incubation. Although it is well established that individual fleas of many species are capable of completely freeing themselves of *P. pestis*, the effect of external temperature upon this mechanism has not yet been clarified. The data in Table 2 show that *X. cheopis* held at 30°C. had a very high infection potential when compared with the groups of fleas held at lower temperatures. Since the data are based upon infection at death of the flea, it is apparent that a substantial percentage of the fleas held at a high temperature did not clear themselves. This reluctance to clear may also be a species characteristic since Eskey and Haas (1940) and Burroughs (1947) have shown that a smaller percentage of *X. cheopis* cleared when compared with other species under the same conditions.

When the blocking-survival potentials are calculated and the vector indices are derived (Table 3), the values are arranged somewhat differently from those showing vector efficiency (Table 2). The longest period of extrinsic incubation occurred at the lowest temperature, and the shortest duration of life after blocking occurred at the highest temperature. Thus the lower overall vector capacity of those fleas held at 8°C. is reflected in a lower vector index when compared with all but one of the groups below 30°C. The published data of Eskey and Haas (1940) can be calculated to show a blocking-survival potential of 0.13 (at 19°C.); their vector index is about 0.02 because of the very low vector efficiency (0.19).

The blocking-survival potential of the fleas held at 30°C. may be the clue to the low vector capacity of this group. As indicated above, the infection potential was high and, likewise, the blocking potential level cannot account for the poor vector efficiency. The blocking potential was on the low side, but it was on a par with those fleas held at about 21°C. in the experiments of Wheeler and

TABLE 4

The plague vector efficiencies of various wild rodent flea species compared with Xenopsylla cheopis*

Flea Species	Approx. Mean Temp. (°C)	Test Date	No. Used	No. Infect.	No. Block.	No. of Trans.	Infect. Poten.	Block Poten.	Trans. Poten.	Vector Efficiency
Xenopsylla cheopis	21	9/48	102	79	56	50	0.77	0.70	0.89	0.48
Epitedia wenmanni wenmanni × testor	17	9/48	135	48	9	6	0.34	0.18	0.66	0.04
Megabothris abantis	21	9/49	12	8	3	1	0.66	0.37	0.33	0.08
M. clantoni johnsoni	20	9/49	15	11	2	0	0.73	0.18	0	0
Monopsyllus exilis kansensis	17	9/48	19	8	2	2	0.42	0.25	1.00	0.11
M. wagneri wagneri	21	9/49	61	18	1	1	0.29	0.05	1.00	0.01
Thrassis fotus	17	8/48	21	10	3	2	0.47	0.30	0.66	0.09
T. bacchi johnsoni	21	9/49	34	18	6	6†	0.52	0.33	1.00	0.17
Hystrichopsylla sp.	21	10/49	29	21	5	6	0.72	0.24	1.20	0.20
Leptopsylla segnis	20	10/47	56	32	0	0	0.57	0	0	0
Atyphloceras multidentatus	21	10/49	80	17	0	0	0.21	0	0	0
Meringis shannoni	20	9/49	26	5	0	0	0.19	0	0	0
Catallagia decipiens	20	10/49	13	2	0	0	0.15	0	0	0

* All transmission experiments were conducted with albino laboratory mice; see Table 5 for additional data.

† One flea transmitted plague to 8 mice as follows: 10/24, 1; 10/25, 2; 10/26, 3; 10/27, 2; if only one infective feeding per day is taken as the basis for deriving the transmission potential, then the above values result; however, if the additional 4 plague transmissions by this flea are taken into account, then the transmission potential is 1.66 and the vector efficiency is 0.28.

TABLE 5

Extrinsic incubation, survival, and vector indices of plague-infected wild rodent fleas compared with Xenopsylla cheopis

Flea Species	Ext. Incubation (days)		Death After Block. (days)		Blocking-Survival Potential*	Vector Index†
	Range	Mean	Range	Mean		
Xenopsylla cheopis	11–49	22.0	1–11	3.0	0.13	0.06
Epitedia wenmanni wenmanni × testor	15–61	30.0	2–5	3.3	0.11	0.004
Megabothris abantis	9–47	28.0	1	1.0	0.03	0.002‡
M. clantoni johnsoni	4–5	4.5	2–5	3.5	0.77	0
Monopsyllus exilis kansensis	12–13	12.5	2	2.0	0.16	0.01‡
M. wagneri wagneri	—	49	—	2	0.04	<0.001‡
Thrassis fotus	15–28	21.6	1	1.0	0.04	0.003‡
T. bacchi johnsoni	8–46	24.7	1–4	2.2	0.08	0.01
Hystrichopsylla sp.	7–25	16.0	1–3	1.6	0.10	0.02
Leptopsylla segnis	—	—	—	—	0	0
Atyphloceras multidentatus	—	—	—	—	0	0
Meringis shannoni	—	—	—	—	0	0
Catallagia decipiens	—	—	—	—	0	0

* Mean day of death after blocking/mean day of blocking after infectious meal.

† Vector efficiency × blocking-survival potential.

‡ Of questionable significance since too few cases were available on which to base the blocking-survival potential.

Douglas (1941, 1945). Although the extrinsic incubation period was quite short, survival of the fleas after blocking was also very brief. Thus the blocking-survival potential was unusually low. On this basis, it is suggested that the extremely short survival of the infective fleas did not allow for many transmission opportunities, thus producing the lowest transmission potential of all the groups listed.

It is difficult to account for the great variation in vector indices for those groups of fleas held at from 20°–22°C. Perhaps differences in techniques may have been responsible. The fleas used by Wheeler and Douglas (1941, 1945) and by Burroughs (1947) were confined to individual tubes even while feeding, whereas those used in the other tests, shown in Table 2, were allowed to come out of their tubes and to feed freely on the abdomen of the mouse. These circumstances, however, do not seem convincing enough to explain why the incubation period of *P. pestis* in Burroughs' *X. cheopis* should be so similar to the incubation period in fleas held at a temperature which was higher by about 9°C. The effect of strain differences seems a remote possibility since all of the *X. cheopis* came from the San Francisco Bay region.

It is clear that the problem of temperature in relation to the plague vector capacity of fleas needs further clarification since, as Park (1941) has stated, "... the number of infective fleas ... is of prime importance, and this depends perhaps entirely on climatic conditions". From the epizootiological point of view, more studies are needed to provide factual data regarding seasonal fluc-

tuations of temperature and humidity in the microclimatic conditions affecting rodent burrows. It may be in the burrow and nest that the major conditions which limit the transmission of plague by fleas from rodent to rodent are to be found.

Table 4 summarizes the data for determination of the plague vector efficiencies of 11 species of wild rodent fleas, the common mouse flea, and the oriental rat flea, *Xenopsylla cheopis* for comparison. A glance at the column showing vector efficiency values shows the superiority of *X. cheopis* in the biological transmission of plague to laboratory mice. It should be borne in mind that, in some instances, few fleas were available and the results in those cases may represent merely a fair approximation of experimental vector capacity.

Table 5 summarizes data regarding the periods of extrinsic incubation of the plague bacilli in the fleas, the survival of the fleas after blocking, and the vector indices. Tables 4 and 5 should be consulted together.

Epitedia wenmanni wenmanni × *testor*

The vector capacity of this species has not heretofore been reported. The data indicate a plague transmission efficiency equal to that of the inefficient strain of *D. montanus* held at 21°C. (Table 1). However, the average temperature was about 4°C. lower than that for *D. montanus* and this may have depressed somewhat the blocking rate. The vector index is slightly below that of *D. montanus* primarily because the extrinsic incubation period was almost twice as long. This may be the direct result of the lower temperature affecting the infection in this flea.

Megabothris abantis

Burroughs (1947) reported the vector efficiency of this species as 0.05. This figure is very close to the vector capacity reported here. In Burroughs' work with this species, the mean extrinsic incubation period was shorter, and the time of survival after blocking was longer than in the present experiment. Thus Burroughs' figures give a blocking-survival potential of 0.23 and a vector index of 0.01, both of which exceed the present values. Although few fleas were available, the present data suggest that this flea species is a capable biological vector. Its possible importance in wild rodent plague transmission is further strengthened by Burroughs' (1947) positive results with mass transmission experiments using the vole *Microtus* sp.

Megabothris clantoni johnsoni

Although no plague transmissions were obtained with this species, the small number of fleas used should be taken into account. Burroughs (1947) used two lots of *M. abantis* and obtained no transmissions in one lot of 48 fleas, while another lot of 27 fleas gave 4 transmissions. Thus the negative results with *M. clantoni johnsoni* may not be significant. As a matter of fact, both the infection potential and the blocking-survival potential for this species were higher than that shown by *M. abantis*. It is of interest that the related *M. clantoni*

clantoni, taken from *Lagurus curtatus* in Lincoln County, Washington, were found to be plague positive (Pub. Health Rep., 1951b).

Monopsyllus exilis kansensis

This species gave the third highest vector efficiency value of the wild rodent fleas tested. Experimental testing of the vector capacity of this flea has not heretofore been reported. Although the survival of this flea after blocking was fairly poor, the period of extrinsic incubation was next to the shortest of any of the flea species tested. Thus the vector index was fairly high when compared with the other wild rodent fleas. It is of interest that plague positive pools of this species have been taken from the grasshopper mouse, *Onychomys leucogaster*, in Texas (Miles *et al.*, 1952).

Monopsyllus wagneri wagneri

Of all the species that transmitted plague, this flea gave the lowest values for both the vector efficiency and the vector index. Eskey and Haas (1940) were unable to obtain any plague transmission to guinea pigs with this species. They used much fewer fleas than in the present instance and the poor success obtained here suggests that a large number must be used in order to obtain one flea that may become infective and transmit. In nature, this flea has been found infected on *Peromyscus truei* in New Mexico (Pub. Health Rep. 1951a), and on several species of *Peromyscus* and on *Reithrodontomys megalotis* during a plague epizootic in New Mexico (Holdenried and Morlan, 1955).

Thrassis fotus

This species gave a vector efficiency close to that of *M. abantis*. The similarity was also shown by the blocking-survival potential and the resulting vector index. Although the vector capacity of this flea has not been reported previously, Wayson (1947) lists it as having transmitted plague experimentally (unpublished studies by F. M. Prince). This species has been found naturally infected on a number of occasions as summarized by Pollitzer (1954).

Thrassis bacchi johnsoni

This species was shown to be one of the most efficient plague vectors and also had one of the highest vector indices. The vector capacity of this flea has not formerly been reported, although work with related species has been published by Eskey and Haas (1940) and by Prince (1943a).

Eskey and Haas (1940) tested 7 different *Thrassis* species as vectors to guinea pigs and obtained results which give vector efficiencies ranging from zero to 1.10 with an average vector efficiency of 0.19. Prince (1943a), working with the related subspecies *T. bacchi bacchi* from S. Dakota, obtained a vector capacity to guinea pigs of approximately 0.17. The remarkable similarity of Prince's results to those in the present report is notable. *T. bacchi johnsoni* also has been found presumptively infected with plague in nature (Pub. Health Rep. 1950). The confused taxonomy of the *T. bacchi* group has recently been clarified by

Stark (1955), a task made even more imperative because of the potential importance of these subspecies to wild rodent plague.

Hystrichopsylla sp.

This species gave the highest vector efficiency and vector index of all the wild rodent fleas reported here. Its vector capacity is here reported for the first time. The potential importance of this flea is emphasized by its consistent involvement in a wild rodent plague enzootic-epizootic area where its natural plague infection rate has been studied (Quan and Miles, unpublished). The negative results obtained by Eskey and Haas (1940) with the related species *H. dippiei* may be discounted inasmuch as only 5 fleas were used. On the other hand, Prince (unpublished, see Wayson, 1947) has obtained plague transmission to white mice with *H. dippiei*.

Leptopsylla segnis

This flea is generally considered to be a very poor plague vector and of little epidemiological importance (Hirst, 1953; Pollitzer, 1954). The present negative results support this view, as do the negative results of Eskey and Haas (1940) who used guinea pigs. On the other hand, negative results with individual fleas should not be taken as final proof that any species of flea is incapable of plague transmission. The mass transmission experiments with *Malaraeus telchinum* (Burroughs, 1944, 1947) is a case in point.

Atyphloceras multidentatus

Negative results were obtained with this species even though, like *L. segnis*, sufficient individuals became infected with *P. pestis* to provide at least a statistical basis for the occurrence of blocking. Eskey and Haas (1940) also obtained negative results, but these were discounted because very few fleas were used. Prince (unpublished, see Wayson, 1947) obtained plague transmission with this species, using white mice. Further light has been shed upon the potential importance of this species by the findings of Quan and Miles (unpublished) that *A. multidentatus* was naturally infected with plague in an enzootic area involving small wild rodent species.

Meringis shannoni

This species also proved negative for transmission of plague, and this is the first report on tests of its vector capacity. In the past, it has been found together with other wild rodent fleas in pools which proved to be plague-positive on inoculation into guinea pigs (Pollitzer, 1954).

Catallagia decipiens

The negative results obtained here agree with the results obtained for *C. wymani* by Eskey and Haas (1940). However, the infection potential for *C. wymani* was over twice that for *C. decipiens*. This flea has been reported in plague-positive pools together with other wild rodent fleas (Pollitzer, 1954).

SUMMARY AND CONCLUSIONS

Experimental studies are reported on the plague vector capacity of 13 different flea species compared with *Xenopsylla cheopis*. Of 12 wild rodent fleas tested, vector efficiency values of the following 8 species are presented for the first time: *Epitedia wenmanni wenmanni* × *testor*, *Megabothris clantoni johnsoni*, *Monopsyllus exilis kansensis*, *Thrassis fotus*, *Thrassis bacchi johnsoni*, *Histrichopsylla* sp., *Meringis shannoni*, and *Catallagia decipiens*.

None of the species tested approached the high vector efficiency of *X. cheopis*. Of the wild rodent fleas used, *Thrassis bacchi johnsoni* and *Hystrichopsylla* sp. had the highest vector efficiencies. The overall vector capacity of these two species, as shown by their vector indices, was much better than any of the other species and approached the value shown for *X. cheopis*. Confirmation was obtained of the vector capacity of some flea species used in these tests and in tests by other workers.

Experiments with the ground squirrel flea, *Diamanus montanus*, showed this species to be a very poor vector under laboratory conditions. These results confirmed former work, and tended to support the hypothesis of Burroughs (1947) that biological strain differences in this species may account for the finding of both low and high plague vector capacity in fleas from different locations.

A mean temperature of about 8°C. was apparently responsible for the prevention of proventricular blocking in *D. montanus*, although the infection potential under these conditions was quite comparable to that of fleas held at higher temperatures.

The plague vector capacity of *Xenopsylla cheopis*, held at an average temperature of about 21°C., was close to that shown by other studies under similar thermal conditions. At mean temperatures of about 8°C. and 20°C., surprisingly high vector efficiencies were obtained. The lowest values were obtained at a mean temperature of 30°C. When the blocking-survival potentials and the vector indices were calculated, the overall plague vector capacity of *X. cheopis* kept at from 20°C. to 22°C. was generally better than that shown by the fleas maintained at 8°C. The poor survival, after blocking, of *X. cheopis* held at 30°C., probably accounts for their very poor transmission potential. A major effect of temperature was apparently its influence upon the extrinsic incubation period of *Pasteurella pestis* in the flea. With *X. cheopis*, the average incubation period at 8°C. was 34.4 days; at from 20°–22°C., it was 18.6 days; and at 30°C. it was 11.9 days.

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SOME NEW COMPARATIVE INVESTIGATIONS ON THREE *PHYSOPSIS* BORNE SCHISTOSOMES: *SCHISTOSOMA HAEMATOBIUM*, *S. BOVIS* AND *S. INTERCALATUM*[1]

J. SCHWETZ

Honorary Professor, University of Brussels

Although *Schistosoma haematobium* and *S. bovis* have been known for a long time as compared with *S. intercalatum* Fisher, 1934, all three are old acquaintances to which I devoted three papers in 1951 (Schwetz, 1951, a, b, c). Even the best known and most investigated schistosome, *S. mansoni*, is the subject of scores of new studies every year and is still much discussed not only as to details but fundamentals as well.

Much practical information (as contrasted with textbook information) on the three schistosomes in this study was gathered, before 1952, from two main sources: (1) from a focus of vesical bilharziasis in Uganda, which on investigation proved to be a mixed focus of *S. haematobium* and *S. bovis* (Schwetz, 1951a); (2) from material obtained at Stanleyville consisting of human stools and *Physopsis* snails infected with *S. intercalatum*, which we studied in our Brussels laboratory (Schwetz, 1951b). But in spite of the new data gathered and described at that time we felt that there was much to be done to elucidate certain seemingly contradictory and obscure facts relating to the etio-epidemiology of this triad. We were handicapped by the fact that our former studies were conducted in a mixed focus[2] of two schistosomes and by the necessity of having to study material which was sent to us rather than material which we had collected in the field.

Bearing these shortcomings in mind, we decided it would be beneficial to pursue our investigations of these three schistosomes in their distinct and individual foci, the location of which we had determined on our previous sojourns in Africa. We were able to carry out the desired investigations during our last two long missions in the Belgian Congo, in 1952 and 1954–55.

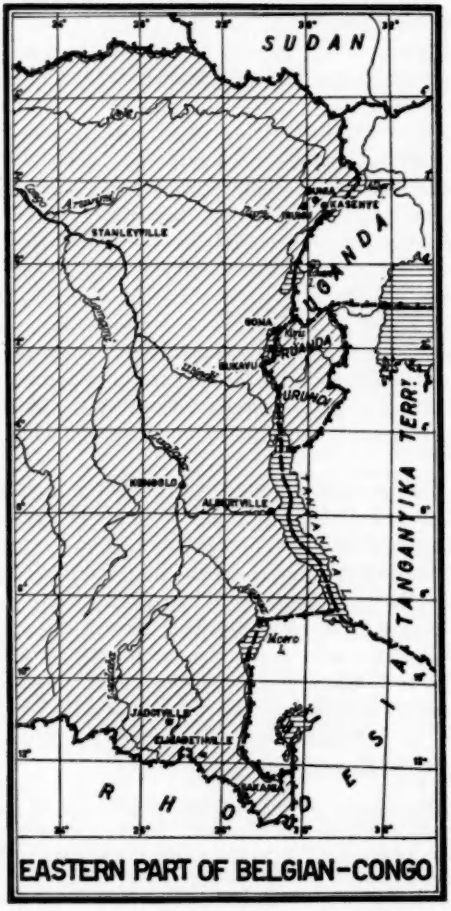
As might be expected, *S. haematobium* proved to be a very strange and capricious species, not only when compared with *S. mansoni* but also in comparison with its two "congeners," *S. bovis* and *S. intercalatum*, which also use *Physiopsis* as an immediate host. In order to emphasize the pecularities in the transmission of *S. haematobium* as compared with that of the other two species, we shall start with a discussion of *S. bovis*.

[1] This investigation sponsored by the Antibilharzia Mission of the Belgian Colonial Office and the University of Brussels with a grant from the Comité Special du Katanga.

[2] We define a "mixed focus" as any focus where two schistosomes, such as *S. haematobium* and *S. bovis*, are transmitted by one and the same mollusc; but not a focus where two schistosomes are transmitted by two separate molluscs such as a focus of *S. bovis* and *S. mansoni* or of *S. mansoni* and *S. intercalatum*.

BOVINE AND OVINE BILHARZIASIS CAUSED BY *S. BOVIS* IN THE HAUT-ITURI, BELGIAN CONGO

This is an undulating savanna region at a mean altitude of 1,000 to 1,200 meters, overlooking Lake Albert (see Map 1). The watercourses of the entire region contain both *Planorbis pfeifferi* and *Physopsis africana*. Intestinal bilharziasis is prevalent in this region. During our investigations in 1949 we found a high percentage of the native population of Bunia and Irumu infected with *S. mansoni* and some of the *Planorbis* in the rivulets of these communities shedding cercariae. We found no cases of vesical bilharziasis, nor could we find any



Map 1

infected *Physopsis* (Schwetz, 1951d). Examinations of the local inhabitants of the two communities as well as examinations of the fluviatile molluscs were repeated in March and April, 1955, with identical results.

The region of Haut-Ituri is a cattle raising center. Examination of cattle by the Veterinary Service over a period of years have revealed various helminthic infections such as *Fasciola gigantica*, *Cysticercus bovis* and *S. bovis*. We did post-mortem examinations on 16 cattle at the Bunia and Irumu slaughter-houses and found *S. bovis* in 11 (70%). Adult worms were numerous in the mesentery and less numerous in the liver. Eggs were found in the liver and in scrapings of the rectal mucosa. In contrast, we were able to demonstrate the eggs of *S. bovis* in the feces of only two animals with acute infections. Failure to find eggs in the other cases was doubtless due to the difficulty of hunting for them in the great mass of feces, a task as illusory as looking for a needle in a haystack. It must be concluded that the diagnosis of bilharziasis in cattle *in vivo* is problematical.

One may inquire why none of the *Physopsis* from the watercourses of Bunia and Irumu was found to be shedding schistosome cercariae in view of the high infection rate in the cattle. These cattle were not bred locally but were imported from neighboring regions, and we therefore undertook to examine snails from the brooks watering the meadows of the breeding centers. *Physopsis* was found shedding cercariae in the breeding centers in the neighborhood of Bunia and Irumu, but we were unable to find any parasitized *Planorbis*.

Although we have given the details of our recent investigations on bovine bilharziasis elsewhere (Schwetz, 1955) we shall present here a few of our results on the experimental transmission of *S. bovis*. This information is of special interest when compared with similar studies on *S. intercalatum* and more particularly *S. haematobium*. We collected 40 *Physopsis* (all shedding numerous cercariae) from the Amande brook and on 22–4–55 they were sent from Irumu to Brussels, reaching Brussels on 26–4–55. Thirty of the snails were living and 14 were used to infect mice with the following results:

26– 4–55	14 *Physopsis* with cercariae used to infect mice
22– 6–55	2 mice showed eggs in feces on the 57th day
16– 8–55	5 of 40 infected *Physopsis* shed cercariae on the 56th day
17–10–55	2 mice showed eggs in feces on the 61st day
	etc . . . etc . . .

We can conclude from this series of transmissions that in *S. bovis* infections the eggs make their appearance in the feces of mice and cercariae are shed by *Physopsis* between the 50th and 60th day after infection. This compares favorably with the results obtained in *S. mansoni* and *S. intercalatum* infections but differs markedly from similar studies on *S. haematobium* as will be pointed out later.

Details relative to the virulence of *S. bovis* infection in mice are presented below.

1. *Bunia*

28–3–55 Five mice were exposed for one hour to numerous cercariae shed by 5 *Physopsis* from the Mybio river. One mouse died the next day.

27–5–55 (59th day) 3 mice with eggs in feces.

13–6–55 Two other mice positive, one of which was ill and died the following day. Postmortem examination revealed the following: liver–pigment, mature eggs and adult worms; mesentery—several pairs of adult worms; scrapings of mucosa and feces both containing mature eggs.

23–6–55 (80th day) 4 remaining mice positive (2 mice died in transit and were not examined).

2–8–55 (127th day) 2 surviving mice negative

2. *Irumu*

20–4–55 6 mice were exposed for 40 minutes to numerous cercariae shed by 5 *Physopsis* from Amande brook.

13–6–55 (54th day) One mouse with eggs in feces.

17–6–55 (58th day) Two other mice positive; one with bloody diarrhea, sacrificed. Postmortem examination revealed: Liver—numerous eggs and adult worms, Mesentery—11 pairs. Scrapings of mucosa and feces—numerous eggs.

23–6–55 (64th day) Fourth and fifth mice were positive.

14–7–55 (85th day) Two more positive mice died. Eggs were found in the liver, mesentery, mucosa and feces. The three surviving mice remained negative at the final examination on 17–8–55 (129th day).

3. *Infection of* Rattus rattus

21–4–55 One *Rattus rattus* was exposed for 30 minutes to cercariae shed by 5 *Physopsis* from the Amande river. The rat was not examined until 25–6–55 (60th day) when numerous typical eggs of *S. bovis* were found in the feces. The rat died on the 80th day and examination revealed numerous adult worms of both sexes in liver and mesentery as well as many eggs in the liver and feces.

SCHISTOSOMA INTERCALATUM

The savanna of Haut-Ituri marks the eastern border of the equatorial forest which stretches along the Congo River down to its mouth. Stanleyville (Fig. 1), an important European and native center, astride the Congo River, is approximately 400 miles west of Bunia and Irumu. There is no large scale cattle raising in the region of Stanleyville, the only local cattle belonging to small dairy farms. The cattle for the local meat market are brought from other regions, Haut-Ituri being one of them. After our study of *S. bovis* and *S. mansoni* in the district of Haut-Ituri, we went to Stanleyville (May, 1955) to investigate the problem of *S. intercalatum*, hoping to fill in many of the blanks in our knowledge of this form of intestinal bilharziasis, particularly in the area of Stanleyville and

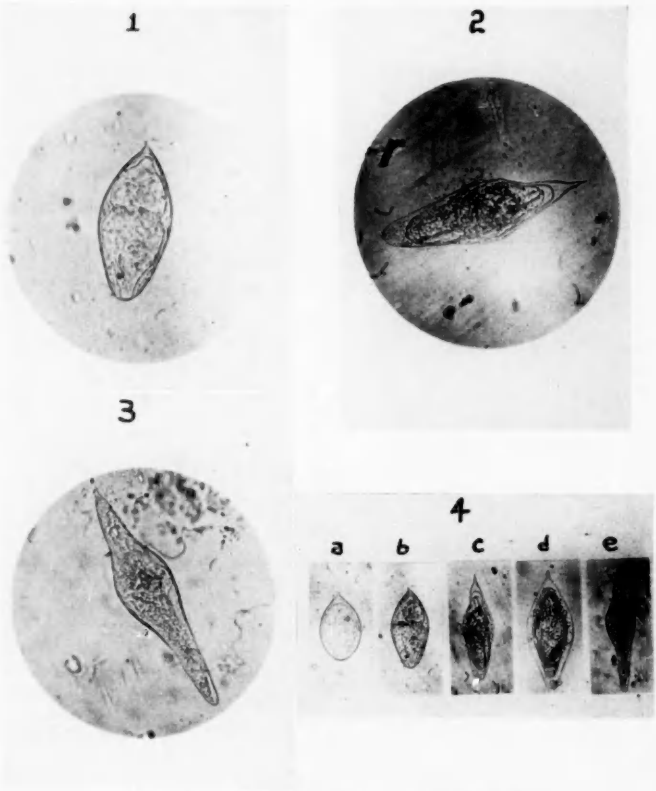


FIG. 1. Egg of *Schistosoma haematobium* (420 ×)
FIG. 2. Egg of *Schistosoma intercalatum* (420 ×)
FIG. 3. Egg of *Schistosoma bovis* (420 ×)
FIG. 4a. Egg of *Schistosoma haematobium* (Loro, Uganda): round form
FIG. 4b. Egg of *Schistosoma haematobium* (Kongolo, Belgian Congo): oval form
FIG. 4c. Egg of *Schistosoma intercalatum* (Stanleyville, Belgian Congo)
FIG. 4d. Egg of *Schistosoma mattheei* (Rhodesia)
FIG. 4e. Egg of *Schistosoma bovis* (Bunia, Belgian Congo)

Yakasu.[3] The blanks in our knowledge of this schistosome are: (1) the failure of Fisher (1934) to complete the life cycle by infecting *Physopsis* with the miracidia hatched from eggs. In our previous work we also started our cycle with naturally infected *Physopsis*; (2) The morphological identification of *S. intercalatum* eggs; (3) The clinical aspects of the disease; (4) The susceptibility of sheep for *S. intercalatum*.

[3] It was in Yakusu, 10 miles from Stanleyville, that Fisher (1934) conducted his studies on *S. intercalatum*.

As pointed out above, Fisher failed to infect *Physopsis* with miracidia hatched from eggs excreted by mice, which he had infected with cercariae shed by naturally infected *Physopsis*. It is well known that it is more difficult to infect *Physopsis* than *Planorbis*, which explains why many workers including Fisher have failed to infect the former. However, the explanation given by Fisher is so unusual and out of keeping with the principles governing the experimental transmission of schistosomiasis that we feel it should be reproduced textually here, any comment being superfluous. "Although it is probable that this lack of success was largely due to some undiscovered error in technique, yet it is possible that these molluscs living as they do in water which must be heavily charged with miracidia, have developed a certain degree of species resistance to infection" (Fisher, 1934, p. 286).

Although we had successfully infected *Physopsis* and thereby completed the life cycle of *S. intercalatum* (Schwetz, 1951c), our work is subject to the same criticism as Fisher's in that our cycle also began with naturally infected *Physopsis* and not with eggs from human feces. We therefore took this opportunity to rectify the flaws in our previous experiment. Three bottles of human feces abounding in eggs of *S. intercalatum* were sent to Brussels on 18–5–55. On their arrival three days later, numerous miracidia had hatched from the eggs, and these were used to infect *Physopsis*, which shed cercariae 56 days later (16–8–55). These cercariae were used to infect 2 mice, which showed eggs in the feces on the 57th day (12–10–55).

In 1951 our experimental life cycle of *S. intercalatum* had given the following results.

I. 1. Cercariae from naturally infected *Ph. africana* were used to infect 4 mice which showed eggs in the feces on the 52nd day.
   2. Of 22 exposed *Ph. africana*, 7 had become positive by the 69th day.

II. 3. 10 exposed mice became positive between the 49th and 57th day.
   4. 50 *Ph. africana* developed one infection only, manifest on the 96th day.

Thus it seems that the snails are not as readily infected as the mice. Upon continuing our study of the life cycle in 1955 we found even more irregularities than in our previous study in 1951.

MORPHOLOGICAL IDENTIFICATION OF *S. INTERCALATUM* EGGS

We shall be very brief on this subject although the theoretical aspects could be discussed endlessly. Eggs of *S. haematobium* recovered from various foci are not identical in shape. We have seen round eggs (in Aloro, Uganda) (Fig. 4a) as well as oval eggs (Figs. 1 and 4b) and even some elongate eggs that resemble eggs of *S. intercalatum* (in Jadotville). We have also observed variations in the eggs of *S. bovis* (Figs. 3 and 4c), some being "a little shorter" or "less spindle shaped." In spite of the variations common in schistosome eggs, anyone who has seen the eggs of *S. intercalatum* (Figs. 2 and 4c) with their elongate shape and sharp, long spine, either from human stools or the feces of mice, will agree that there is absolutely no basis for confusion.

Clinical aspects of the disease. We had heard that the intestinal bilharziasis of

Stanleyville was less severe and more amenable to treatment than the usual intestinal bilharziasis caused by *S. mansoni*. We spent eight days examining some of the native inhabitants on a coffee plantation there, in an attempt to elucidate this matter. The results of our examinations were:

	No.	Positive	
Adult males	83	25	30.1%
Adult females	83	26	31.3%
Boys	46	29	63.0%
Girls	39	17	43.0%
	251	97	38.6%

All of the infections were *S. intercalatum* with the exception of a few in men originally from Haut-Ituri who were infected with *S. mansoni*. We found quite a number of cases with bloody stools containing numerous eggs, and many others gave histories of divers gastro-intestinal symptoms such as diarrhea, abdominal pain, etc. We believe therefore that assertions of the relative benignity of *S. intercalatum* infections are unfounded. Besides, the severity of *S. mansoni* infections varies in different foci, ranging from very benign to extremely virulent forms of the disease.

Susceptibility of sheep to S. intercalatum. This important subject, which is very interesting from the biological viewpoint, was dealt with very briefly by Fisher (1934) and only *en passant*. The following excerpts from Fisher are his only references to this subject: (a) "Description is based on material obtained from experimentally infected mice and one sheep" (page 278); (b) "No natural infections were found among the few sheep and goats kept by the natives. One male sheep was successfully infected by giving cercariae by mouth" (page 283). Fisher failed to mention the nature of the disease, the presence or absence of eggs in the feces or whether only adult parasites were found in the viscera. He does not give the time interval between exposure and patency of the infection, but these details are of utmost importance in this case, since, from his terse description one might suspect that the schistosome involved was *S. mattheei* (Fig. 4d) which is morphologically similar to *S. intercalatum*. Since Stanleyville is not too distant from Haut-Ituri, and we found cattle infected with *S. bovis* in the slaughterhouses of Stanleyville, it is possible that one might also find a sheep introduced from Haut-Ituri and infected with *S. bovis*.

Due to the lack of specific information on *S. intercalatum* infections in sheep we proceeded to study the problem. For this purpose we purchased a great ram, originally from Haut-Ituri; a young ewe from Stanleyville and a young goat also from Stanleyville. Our experiment was carried out with the assistance of native personnel placed at our disposal by the local Hygiene Department. Since a large number of cercariae were needed for the experiment, we collected thousands of *Physopsis* from the Kitofu River, where we had previously found a large colony of infected snails.

The exposure of the animals was done over a four-day period. Each day the animals were kept without food or water until noon or 2:00 p.m., when they were allowed to drink water, with or without pieces of manioc, containing freshly shed cercariae from our test tubes. As a rule the sheep drank more water than the goat. The exposure of the animals was completed on 28–6–55 just before we returned to Europe. We left the three animals with the director of the medical laboratory in Stanleyville, who graciously consented to care for them and send us the necessary material from the animals. The results of our experiment are based on information supplied by Dr. Courtois, and on the examination of material he sent to us in Brussels.

The ram, as we said, came from Haut-Ituri. On 19–8–55 (52 days after exposure) examination of the feces disclosed one *S. bovis* egg. On 24–8–55 (57 days after exposure) the ram became ill and died of pneumonia the following day. Schistosome eggs were not found in the feces sent to us in Brussels although more than 50 preparations were examined, but among the helminths found in the liver and sent to us were one female and several male schistosomes. The female, which was not flattened, contained typical eggs of *S. bovis*. Thus the ram, infected with *S. bovis*, died of intercurrent disease.

The results of the examination of the goat and the ewe, both from Stanleyville, may be summarized as follows.

Date of examination	Ewe	Goat	Remarks
19– 8–55, 52nd day	+ *S. intercalatum*	0	Blood in the feces of the ewe.
23– 8–55, 56th day	+ *S. intercalatum*	0	
30– 8–55, 63rd day	+ *S. intercalatum*	0	Numerous eggs of *S. intercalatum* in the preparations we received.
5– 9–55, 68th day	+ *S. intercalatum*	0	
27– 9–55, 89th day	+ *S. intercalatum*	+ *S. intercalatum*	Rare eggs of *S. intercalatum* in the feces of the goat.
30– 9–55, 92nd day	+ *S. intercalatum*	+ *S. intercalatum*	
4–10–55, 98th day	+ *S. intercalatum*	+ *S. intercalatum*	
9–10–55, 103rd day	+ *S. intercalatum*	+ *S. intercalatum*	

Both the goat and ewe were infected with *S. intercalatum* as a result of drinking water containing cercariae shed by naturally infected *Physopsis*. Since the goat drank less water than the ewe the signs of the infection appeared later than in the ewe. The eggs found in the feces of both animals were characteristic of *S. intercalatum* and as typical as eggs from human stools or the feces of mice.

VESICAL BILHARZIASIS

When in the course of a field study on schistosomiasis, one passes from a focus of *S. mansoni* to a focus of *S. haematobium*, one is astonished at the great difference between the two. Everything is clear and simple in the evolution of *S. mansoni*, while all that concerns *S. haematobium* is obscure, irregular and even contradictory. This difference is particularly striking in foci common to

both species, that is, in localities affected with both intestinal and vesicle bilharziasis. We can illustrate this with several examples.

Jadotville (Katanga) is a focus of both intestinal and vesical bilharziasis, the latter being predominant (Schwetz, 1953d). There are numerous *Planorbis* and *Physopsis* in Jadotville, which we regularly collected and examined. Out of the first 124 *Planorbis* collected 38 (30.6 per cent) were parasitized. On the other hand, it was only after we had collected and examined 1,517 *Physopsis* over a period of eight days that we were able to find the first parasitized snail, which shed no more than three cercariae.

The difference in the infection of laboratory animals with *S. mansoni* and *S. haematobium* is even more spectacular. In the same focus in Jadotville, mice, infected with cercariae shed by *Planorbis*, began to excrete eggs in the feces on the 50th day, while mice infected with cercariae shed by *Physopsis* did not excrete any eggs for 100 days, and on being killed, disclosed at autopsy only a few male worms or rare degenerate eggs in the liver or rectal mucosa. It is true that in the case of intestinal bilharziasis, we had many *Planorbis* shedding numerous cercariae while in the case of vesical bilharziasis we had few *Physopsis*, sometimes only one or two, shedding a very small number of cercariae, at times only one or two. We attribute the great number of unisexual infections in vesical bilharziasis to the scarcity of cercariae. Whatever the cause, we obtained the same results in Elizabethville, Sakania and other localities where intestinal and vesical bilharziasis were coexistant. This being so, it is practically impossible to infect laboratory-reared *Physopsis*.

To investigate this further, we decided to go to Kongolo, an important focus of vesical bilharziasis with practically no intestinal bilharziasis.[4] We spent May, 1952, investigating the problem in Kongolo and have reported our results in a previous paper (Schwetz, 1953a). Here we shall confine our remarks to a short malaco-schistosomical summary.

The locality of Kongolo, situated on the shores of the Congo River, contains numerous water courses, one of which is the swampy Kangoie. There are several native compounds in Kongolo. In one area we examined 811 children, adolescents of both sexes (between the ages of 7–8 and 12–13), and found 542 (66.7 per cent) infected with vesical bilharziasis. In the largest native compound of Kangoie, the incidence of infection was 80.7 per cent and in another native village, in Misalwe, it reached 90 per cent.

With the exception of a few rare *Planorbis* the mollusc population of Kongolo was composed of three species: *Lanistes sp.*, *Lymnaea sp.* and *Physopsis africana*. The *Physopsis* were abundant. In spite of the fact that a large number of people were found infected and were passing numerous eggs of *S. haematobium* (sometimes revealed by simple urinalysis) we did not find a single infected *Physopsis* out of hundreds examined. Finally we collected three *Physopsis*, shedding very few cercariae, from a small river. These cercariae were used to infect three mice

[4] We say "practically" because there were several cases of intestinal bilharziasis, which originated in neighboring or more distant places and not in Kongolo itself.

which were killed between the 86th and 100th day after exposure. Postmortem examination disclosed several adult pairs of worms; eggs were seen in the uterus of one female, but no eggs were found in the tissues of the animals. Our work in Kongolo was done during the rainy season when the rising waters of the Congo River overran the countryside and backed up in its tributaries. Thus the *Physopsis* we found were probably not from that locality, which would explain the absence of infected molluscs despite the high incidence of bilharziasis. Consequently, we decided to return to Kongolo in the dry season when the waters of the river are low. We were not able to do this until three years later, in January, 1955.

The rainy season had just begun; the rivers and ditches which had been completely dry at the end of the dry season contained a little water and several young molluscs. The banks of the Kangoie could be easily seen since they were less swampy at this time of year. On following the shoreline past the native compound of Kangoie, we came to a series of deep water holes overrun with vegetation and containing numerous *Physopsis* (and *Lanistes*) but no *Planorbis*. These water holes were used by the natives for various purposes—reservoirs for drinking water in the dry season, for clothes washing, for baths and some were used for the maceration of manioc.

No time was lost putting this windfall to good use. We collected 320 *Physopsis* and placed them in 80 tubes, 4 in each tube. Early on the same afternoon we found numerous cercariae in 30 tubes, so that at least 30 *Physopsis* were shedding cercariae, the minimal theoretical incidence being 10 per cent. This is a high incidence of infection for *Physopsis* and the fact that numerous cercariae were being shed by the snails was surprising in view of our past experience.

We thought for a moment that we were about to solve our problem but we soon found a number of obstacles in the way. We were on an ambulant mission, a very useful and even indispensable method of collecting material, but of no value for a thorough and profound study of the material collected. Therefore, we had to find an expedient way of sending our material to a well equipped laboratory. Using the local air facilities, which are indirect and irregular, we sent seven shipments to our Brussels laboratory, with the result that all of the 869 *Physopsis*, 25 per cent of which were shedding numerous cercariae, were dead on arrival in Brussels. Two other shipments, sent to Mr. Standen in London, met the same fate. We therefore had to resort to the following stratagem. We asked the Medical Service in Kongolo to collect large numbers of *Physopsis* in the water holes of Kangoie and send them at once by rail to Albertville, 250 miles away, where we disposed of the dead snails and changed the water. We then took our precious cargo by car 350 miles to Bukavu, where the Hygiene Laboratory, directed by Dr. Gillet, is located and fast planes leave regularly for Europe.

Needless to say, our collection suffered many losses as a result of this long and devious trip to Bukavu, the greatest number of deaths occurring among the infected snails. Thus we could not expect to find the same incidence of infection among the survivors as we did originally in Kongolo. The results of the examination of part of the thousand *Physopsis* which reached Bukavu was as follows:

15–2–55	222 *Physopsis*	4 positive = 2%
16–2–55	222 *Physopsis*	6 " = 3%
17–2–55	222 *Physopsis*	8 " = 4%
Total.....	666 *Physopsis*	18 " = 3%

Although the per cent of positive snails was considerably less than found in the original collections in Kongolo, it was still fair considering *S. haematobium* standards. Furthermore, these snails were shedding numerous cercariae, not just 2 or 3 as we had found previously. It is possible that not all of the positive snails were detected. After several mice had been exposed to cercariae, some positive *Physopsis* were sent to our Brussels laboratory and to Mr. Standen of the Wellcome Laboratory in London. The remaining material was left with Dr. Gillet, who subsequently sent us in Brussels mice infected by the *Physopsis* we had collected.

A few specific results of our study of the mouse infections in our Brussels laboratory and a summary of the results obtained by our distinguished colleagues, Standen and Gillet, will be presented here.

Results obtained in the Brussels Laboratory

1. 15–2–55 Two mice exposed to numerous cercariae shed by 4 *Physopsis* (Kongolo)
   20–5–55 94th day after exposure the two mice, not having shown any eggs in the feces, were killed and examined. A few adult male worms were found in the liver and mesentery of both mice. Diagnosis: unisexual infection.
2. 17–2–55 3 mice exposed to numerous cercariae shed by 15 *Physopsis* (Kongolo)
   23–5–55 95th day. One degenerate egg found in feces of one mouse, other two negative.
   1–6–55 104th day. One mouse died. Postmortem examination revealed rare eggs in liver, none in feces. Two remaining mice positive but only rare degenerate eggs found in feces.
   23–6–55 127th day. One mouse negative; other mouse positive with two viable eggs in feces.
   6–7–55 104th day. Both mice negative.
   11–7–55 145th day. One mouse died, only a few eggs could be found in liver and feces. Miracidia could not be hatched from these eggs.
   27–7–55 160th day. Surviving mouse negative.
   17–8–55 184th day. This mouse killed and autopsied. No eggs or adults found, but pigment seen in the liver.
3. 18–2–55 Two mice exposed to numerous cercariae shed by 10 *Physopsis* (Kongolo).
   23–5–55 94th day. Both mice negative.
   25–5–55 96th day. One degenerate egg found in feces of one mouse.
   8–6–55 108th day. Both mice negative, killed and autopsied. One mouse showed no evidence of schistosome infection. Examination of the second

mouse revealed one degenerate egg in the liver, several adult male and female worms in the mesentery.

It is unnecessary to detail the results of the other experimental infections since they are identical to those described above. Between the 100th and 200th day after exposure the mice are either negative or exhibit rare degenerate eggs in the feces. Suddenly, the feces become bloody, the mouse dies, having either a few degenerate eggs in the feces or none at all. In some mice we found numerous eggs in the liver, most of which were not viable. For example, one mouse survived for 200 days after exposure to cercariae from Kongolo. After the 100th day the results of fecal examinations were variable; at times we found one or two degenerate eggs and at other times no eggs at all. This mouse died of intestinal hemorrhage on the 200th day. We found areas of pigmentation and several clumps of eggs in the liver as well as several pairs of worms, the females with typical eggs in the uterus. No miracidia could be hatched from these eggs.

The information received from our colleagues in London (Mr. Standen) and in Bukavu (Dr. Gillet) on their experience with the *Physopsis* from Kongolo corroborated our own data and conclusions, namely, unisexual infections, delayed infection, passage of non-viable eggs. In short, it was impossible to establish and maintain a stock infection in the laboratory.

We should not have expected our results to be any different in view of the findings of Standen (1949), Moore and Meleny (1954), Kuntz and Malakatis (1955) and our previous experience in Jadotville and Sakania (Schwetz, 1953c and d). However, the existence of a wide focus of vesical bilharziasis in Kongolo, the availability of thousands of *Physopsis* shedding numerous cercariae in Kongoie aroused in us some illusory hopes. Our work and that of others has shown that in contrast to the other schistosomes, it is practically impossible to reproduce the cyclical transmission of *S. haematobium* using the white mouse as the experimental host. The failure is due to the great delay or definitive suspension of the development of the parasites in the body of the mouse, which in turn prevents the appearance of viable eggs in the feces, without which it is impossible to infect additional snails. In nature, the snails are infected by miracidia which hatch from eggs passed in the feces and/or urine and not by eggs lodged in the liver or intestinal mucosa.

The above leads us to comment on the particularly interesting though somewhat bizarre study of Edwards and McCullough (1954) on intestinal and vesical bilharziasis in the Gold Coast. Unlike most workers, Edwards and McCullough succeeded in reproducing simultaneously the cycle of *S. mansoni* with *P. pfeifferi* and the cycle of *S. haematobium* with *Physopsis africana*. In their summary these authors conclude: "*S. haematobium* is capable of attaining the fully matured adult form in white mice within about 70 days of cercariae infection, as compared with approximately 40 days in the case of *S. mansoni* in white mice" (page 177).

A comparison of their two tables on *S. mansoni* and *S. haematobium* shows that the diagnosis of the maturity of the eggs of *S. mansoni* was made by examining eggs in the feces, whereas in the case of *S. haematobium*, the authors

killed the mice and looked for eggs in the liver and intestinal mucosa. The two procedures are quite different, since obviously in nature, *Physopsis* do not penetrate the viscera in quest of eggs with which they might become infected. Occasionally, one may find a female worm containing mature eggs in the uterus on the 70th day, but one does not usually find any free mature eggs until much later and even then, one often finds only degenerate eggs or a unisexual infection. In addition, we were quite amazed at the precocity of the appearance of cercariae as reported by these authors ("32 days for *S. haematobium* and 27 days for *S. mansoni*") since, as a rule, except when artifically heated, the cercariae of *S. mansoni* and even the cercariae of *S. rodhaini* appear only between the 50th and 60th day after infection of the snail. Whatever the case, although we admire the technical skill of Edwards and McCullough, which enabled them to obtain experimental, though artificial, cyclical transmission of *S. haematobium* using white mice, we adhere to our conclusions, confirming those of many wellknown investigators, namely, that the white mouse which is an excellent host for all other schistosomes is unsatisfactory for the transmission of *S. haematobium*.

DISCUSSION

Impressed by the differences in the experimental transmission of *S. mansoni* and *S. haematobium*, we published a small study on the comparative behavior of the *Planorbis-S. mansoni* and *Physopsis-S. haematobium* complexes). Which link is responsible for this difference, the mollusc link, the parasite link or both?

Having succeeded in completing the cycle parasite-mollusc-parasite (or mollusc-parasite-mollusc) for the complex *Physopsis-S. bovis* and the complex *Physopsis-S. intercalatum*, it becomes evident that the difficulties in the completion of the cycle *Physopsis-S. haematobium* are due to the parasite, *S. haematobium*.

It is true that *Physopsis* is more difficult to maintain under experimental conditions than *Planorbis*, and the experimental transmission of *S. intercalatum* and *S. bovis* is more irregular than that of *S. mansoni* and *S. rodhaini*. But these cycles are reproduced, as in nature, using small laboratory animals, particularly the white mouse. The experimental transmission of *S. haematobium* is quite different from that of the other schistosomes, transmissible by either *Planorbis* or *Physopsis*. This fundamental difference is fairly obvious. *S. haematobium* is the only schistosome causing a disease—vesical bilharziasis—which is not transmissible as such to laboratory animals. *S. haematobium* causes vesical bilharziasis, with accompanying passage of eggs in the urine, only in the primates.[5] All of the other schistosomes cause intestinal bilharziasis, transmissible as such to laboratory animals including the white mouse, with ensuing regular passage of viable eggs in the feces.

Thus we can add a new classification of African schistosomes to the preexisting ones, which are all more or less schematic like all classifications in biology.

A. Schistosome causing vesical bilharziasis in human beings, transmissible to

[5] In the experiments of Kuntz and Malakatis (1955), they sometimes observed a penetration of cercariae, but not complete development of vesical bilharziasis.

primates in which the parasite produces identical lesions thus completing its life cycle; also transmissible to small laboratory animals in which it does not produce the same disease and does not complete its life cycle. 1. *Schistosoma haematobium*.

B. Schistosomes causing intestinal bilharziasis in human beings or in animals, transmissible to small laboratory animals in which they complete their life cycle producing intestinal bilharziasis with the passage of eggs in feces. 2. All other schistosomes: *S. mansoni*, *S. rodhaini*, *S. bovis* and *S. intercalatum*.

We can only mention briefly the new taxonomic tendencies which propose to divide *S. haematobium* into sub-species or varieties depending upon the production of purely vesical infections or recto-vesical infections (Amberson and Schwarz, 1953). Other authors propose to ban the term intestinal bilharziasis as inaccurate "in view of the fact that *S. haematobium* infections in many parts of Africa are rectal as well as vesical" (Chesterman, 1954). We should beware of not seeing the forest for the trees; the terms "intestinal and vesical bilharziasis" indicate the place of the lesions, although the schistosomes may pass through or stay in other organs as well. In a study made many years ago (Schwetz, 1951e) we observed that in a region with only vesical bilharziasis, one finds the eggs of *S. haematobium* in the urine, just as in a region with nothing but intestinal bilharziasis *S. mansoni* eggs are found in the feces. However, in localities where the two schistosomes coexist, one may find a few eggs of *S. mansoni* in the urine among numerous eggs of *S. haematobium* as well as an occasional egg of *S. haematobium* in the feces along with many *S. mansoni* eggs. The exceptional penetration of rare eggs from the rectum into the bladder and vice versa, is quite easy to understand in both host sexes, particularly in women.

SUMMARY

The three schistosomes transmitted by *Physopsis* are morphologically identical but differ in the shape of the eggs: *S. haematobium* eggs are round or oval; *S. bovis* long and spindle shaped, *S. intercalatum*, elongate and slender with a long, slender spine. In spite of the similarity of the parasites and the sharing of the snail host they produce completely different diseases. *S. haematobium* causes bilharziasis only in human beings, reproducible experimentally in primates. *S. bovis* causes an intestinal bilharziasis (and occasionally a concomitant vesical bilharziasis) in cattle, sheep and goats which can be experimentally transmitted to small laboratory animals. *S. intercalatum*, found in certain wooded regions, causes an intestinal bilharziasis in human beings which can also be experimentally transmitted to small laboratory animals. In addition, the transmission of *S. intercalatum* to sheep and goats has been confirmed.

Since some ovides in South Africa have been found naturally infected with *S. mattheei*, a species or variety closely resembling *S. intercalatum*, the question arises as to whether we may not be dealing with the same schistosome, varying a little in its morphology in different hosts, human or animal. We shall abstain from giving a definite answer to this question, but we wish to point out that the eggs of *S. intercalatum* found in the feces of our experimentally infected goat

and sheep were morphologically identical to the eggs of *S. intercalatum* found in human feces.

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STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXX. THE OCCURRENCE OF *ECHINOCOCCUS MULTILOCULARIS* LEUCKART, 1863, ON THE MAINLAND OF ALASKA

ROBERT RAUSCH

Arctic Health Research Center, U. S. Public Health Service, Anchorage, Alaska

Alveolar hydatid disease of man is caused by the invasive growth of a larval cestode of the genus *Echinococcus* (Rudolphi, 1801). Since recognition of the etiologic agent by Virchow (1855), the disease has been diagnosed most frequently in southern Europe and Russia. It has been reported in Siberia at least as far east as the Kolyma River (Semenov, 1954). Farther east, on St. Lawrence Island, it is an important cause of morbidity among the Eskimo (Rausch and Schiller, 1956). Barabash-Nikiforov (1938) and Afanas'ev (1941) recorded the cestode from non-human hosts on Bering Island. Apart from these two islands in Bering Sea, alveolar hydatid disease is not known to occur outside Eurasia.[1] No confirmed autochthonous cases have been reported in continental North America.

For many years the identity of the cestode causing alveolar hydatid disease has been a matter of controversy. Only *Echinococcus granulosus* (Batsch, 1786) was recognized in Europe. Its larva usually has the form of a simple, fluid-filled cyst which is quite unlike the alveolar-form larva sometimes observed in man. On St. Lawrence Island, a species of *Echinococcus* distinct from *E. granulosus* was found and described as *E. sibiricensis* Rausch and Schiller, 1954. Since the larva of the cestode from St. Lawrence Island and that causing alveolar hydatid disease in Eurasia were morphologically identical, and since the disease conditions produced were clinically similar, the possibility that the two species were identical was realized (Rausch, 1952). However, the life cycle of the Eurasian form was unknown, and the adult cestode had never been identified.

The life cycle of the Eurasian species has recently been determined by Vogel (1955), and the adult cestode is morphologically identical with *E. sibiricensis*. It is now apparent that the two are conspecific. Leuckart (1863) applied the name *E. multilocularis* to an alveolar larva referred to *Taenia echinococcus* von Siebold [= *E. granulosus* (Batsch, 1786)]; consequently, as pointed out by Vogel (1955), the name *E. multilocularis* Leuckart, 1863, is the valid one on the basis of prior application.

The study of a large collection of adult cestodes of the genus *Echinococcus* from mainland Alaska disclosed specimens from foxes which differed morphologically from the common *E. granulosus*, but which closely resembled the species on

[1] According to a publication received after this paper was submitted (Inukai, T., Yamashita, J., and Mori, H., *J. Fac. Agric. Hokkaido Univ.* **50**: 134–139, 1955), *E. multilocularis* now occurs on the Japanese Island of Rebun. It was apparently introduced with red foxes from the Kurile Islands. The cestode was originally brought to the Kurile Islands with blue foxes imported from the Komandorskii Islands. Infections in man on Rebun were first noted about 10 years after the introduction of the foxes.

St. Lawrence Island (Rausch, 1953). This material has been restudied, and the existence of *E. multilocularis* in mainland Alaska has been confirmed. It is the purpose of this paper to report these findings, with remarks on their epidemiological significance.

Under natural conditions, the adult *E. multilocularis* is found in the small intestine of foxes. Dogs also become infected, however. The larval stage is harbored by microtine rodents of the genera *Microtus*, *Clethrionomys*, and *Lemmus*. Other species can be infected experimentally.

Two species of fox occur in Alaska. The red fox, *Vulpes vulpes*, is found over most of the Territory, while the arctic fox, *Alopex lagopus*, is restricted essentially to tundra regions of northern and western Alaska. The local abundance of both is correlated with the availability of food. Foxes prey extensively upon mouse-like rodents when the latter are abundant and, with avian predators, migrate into regions where the rodent populations are at a high density.

In the last seven years, during which the present work has been carried on, both arctic and red foxes have been most easily obtainable in arctic Alaska, from the Brooks Range north. Most were collected during and following the high population densities of brown lemmings, *Lemmus sibiricus*, of 1949 and 1952–53. A few animals of both species were killed farther to the south (see map, Fig. 1).

Echinococcus multilocularis was found in two of 100 red foxes, one of which was from Point Barrow, and the other from Nunivak Island, off the west coast of Alaska. Four of 94 arctic foxes harbored *E. multilocularis*. Three of these were taken on the Arctic Coast, at Icy Cape and Wainwright. The fourth was killed inland near the north edge of the Brooks Range. With the exception of the last, all were taken where the brown lemming was the only common microtine rodent. This fox had apparently migrated inland in the autumn of 1953, following a decline in numbers of lemmings along the Arctic Coast. It appears probable that the brown lemming was the source of infection for these animals. This is certain in the case of the red fox from Nunivak Island, for no other microtine rodents are known to occur there. Results of experimental infections have shown that the brown lemming is a suitable host for *E. multilocularis* (Rausch and Schiller, 1956).

The writer has not observed naturally infected rodents on the Alaskan mainland; however, only about 2,500, a relatively small number, have been examined. The low rate of occurrence of the adult cestode in foxes indicates that the larval cestode would be found rarely, since a much higher prevalence is expected in the final host. On St. Lawrence Island, the rate of infection in voles (*Microtus*) has not been observed to exceed 20 per cent in years when 76 to 100 per cent of the arctic foxes harbored adult cestodes. The high prevalence of both larval and adult cestodes in their respective hosts on St. Lawrence Island has been a direct result of a sustained high population density of both host species; no comparable situation has been observed on the mainland.

It is important to note that *E. granulosus* has not been recorded from Alaskan foxes, despite the wide distribution of this cestode. At Point Barrow, five of 50 dogs examined harbored cestodes identified as *E. granulosus*. In the Brooks

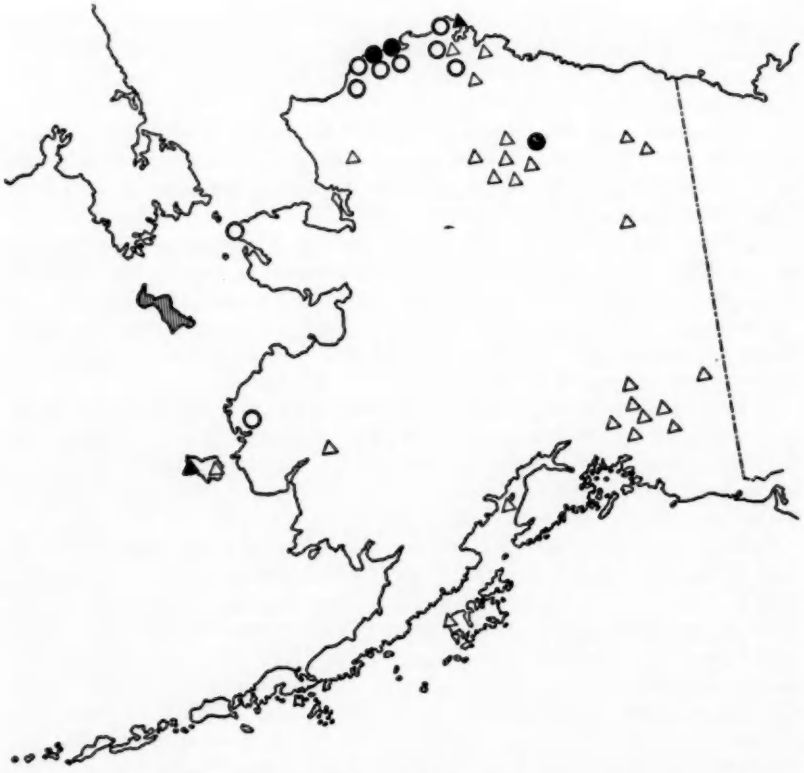

FIG. 1. Map of Alaska showing localities from which foxes were examined. Circles represent arctic foxes, and triangles represent red foxes. Solid symbols indicate foxes harboring *Echinococcus multilocularis*.

Range, 30 of 94 wolves, *Canis lupus*, harbored *E. granulosus*, and it is also common in sledge dogs. Many of the red foxes examined were taken in this region. Sweatman (1952) found no infected foxes in areas where *E. granulosus* occurred in wolves. A review of the literature has disclosed no records of this cestode in foxes in North America.

It is evident from a review of the European literature that there has been much disagreement regarding the importance of the red fox as a final host for *E. granulosus*. Contradictory findings were reported because it was not realized that two species of *Echinococcus* were indigenous in central and southern Europe. Matoff and Jantscheff (1954) infected several young red foxes and found that the life span of *E. granulosus* in this host was very short (25 to 35 days), its development was abnormal, and eggs were never produced. Consequently, it was con-

cluded that the red fox is not a natural host for this cestode, and therefore has no importance in its transmission.

Choquette (cited in Miller, 1953) collected *Echinococcus* sp. from an arctic fox on Bank's Island, in the Canadian Arctic. In view of present knowledge, it is probable that the species involved was *E. multilocularis*. Since the latter is so widely distributed in Alaska, there is no reason to question its occurrence farther east where conditions for completion of its life cycle are equally favorable. *E. multilocularis* appears to be another of a growing list of helminths having holarctic distribution in high-boreal regions.

Unless there is close association with dogs, man is not likely to become infected with either species of *Echinococcus*. There is some risk involved in the handling of infected foxes and wolves by trappers, but this appears to be slight. Continual contact between dogs and man is unavoidable in those regions of Alaska where the sledge dog provides the only practical means of transportation. It is in these regions where human cases are frequently diagnosed.

The larvae of *E. granulosus* are not available to dogs if the animals are prevented access to carcasses or offal of wild ruminants. In the case of *E. multilocularis*, however, dogs become infected when they feed upon rodents containing the larval cestode. Since the feeding upon wild rodents is difficult or impossible to control under usual conditions, infected dogs are to be expected wherever the cestode is present.

It is probable that *E. multilocularis* will be introduced into the United States and southern Canada by dogs imported from arctic regions, if this has not

TABLE 1

A comparison of the biological and morphological characteristics of E. multilocularis *and* E. granulosus

	E. multilocularis	*E. granulosus*
Host-occurrence:	Larval stage in microtine rodents; accidental in man; adult stage in foxes and dogs.	Larval stage in ruminants; accidental in man; adult stage in dogs, wolves, and coyotes.
Larval characteristics:	Alveolar larva which proliferates by exogenous budding, gradually invading and destroying tissue of host.	Larva usually a fluid-filled vesicle, surrounded by a dense connective tissue capsule; no exogenous budding.
Adult characteristics:		
Size of strobila	1.2 to 3.7 mm.	1.5 to 6 mm.
Number of testes	17 to 26 (average 22)	45 to 65 (average 56).
Distribution of testes	From level of genital pore to posterior end of segment.	Both anterior and posterior to genital pore.
Position of genital pore (gravid segments)	Near middle of segment.	Near posterior end of segment.
Uterus	Usually having no lateral branches.	Lateral branches present.

already occurred. With the abundance of susceptible microtine rodents and large numbers of foxes in many agricultural regions, the cestode could become established. If this were to take place, dogs could harbor the adult cestode and the occasional infection of man would be expected. In settled regions, the domestic cat would also be important in the transmission of infection to man. Vogel (1955) determined that *E. multilocularis* develops normally and produces eggs in the cat. Lörincz (1933) found that the adult *E. granulosus* is incapable of maturation and egg production in the cat. Since both species of *Echinococcus* may occur in the United States, their distinguishing characteristics are presented in Table 1. Characteristics of diagnostic value are present in both mature and gravid segments (Figs. 2–5).

An intensive survey to determine more accurately the distribution of *E. multilocularis* in Alaska is now in progress.

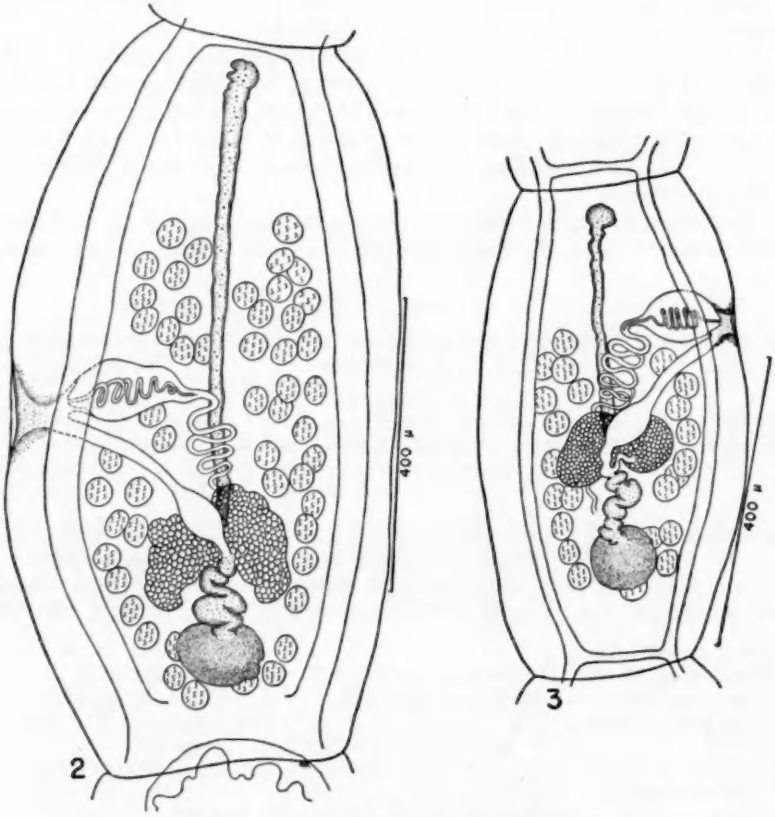

FIG. 2. *Echinococcus granulosus*, mature segment
FIG. 3. *Echinococcus multilocularis*, mature segment

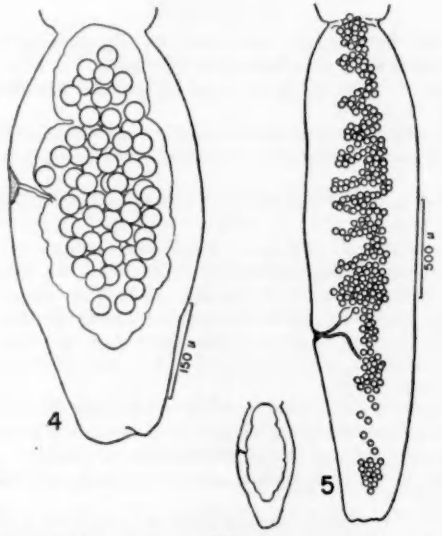

FIG. 4. *Echinococcus multilocularis*, gravid segment

FIG. 5. *Echinococcus granulosus*, gravid segment. Inset shows gravid segment of *E. multilocularis* drawn at same scale.

SUMMARY

Echinococcus multilocularis Leuckart, 1863 (syn. *E. sibiricensis* Rausch and Schiller, 1954), whose larva causes alveolar hydatid disease in man, is reported for the first time from continental North America. The adult cestodes were collected from foxes at four localities in arctic Alaska, and also from Nunivak Island, which lies off the western coast of Alaska near the delta of the Kuskokwim River. *E. multilocularis* is apparently widely distributed in Alaska, and may be expected to occur wherever the predator-prey relationship existing between the arctic and red foxes and microtine rodents, particularly the brown lemming, favors completion of its life cycle.

The controversy of the past regarding the identity of the cestode causing alveolar hydatid disease is now clarified by the recognition of the coexistence of two species, *E. granulosus* and *E. multilocularis*, which differ in host-relationships and morphological characteristics. These species, however, are known to be sympatric only in boreal regions, the distribution of *E. multilocularis* being restricted by the occurrence of suitable intermediate hosts (rodents of the genera *Microtus*, *Clethrionomys*, and *Lemmus*).

Although *E. multilocularis* is known only from parts of Alaska having an abundant mammalian fauna, it is believed that conditions in southern Canada and the United States are favorable enough to permit its becoming established, should it be introduced via infected dogs from the north.

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MOSQUITO COLLECTIONS AND DISSECTIONS FOR EVALUATING TRANSMISSION OF FILARIASIS IN POLYNESIA (TAHITI)[1]

DAVID D. BONNET, JOHN F. KESSEL, JACQUES KERREST AND HERALD CHAPMAN

Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, California and Institut de Recherches Médicales de l'Océanie Française, Papeete, Tahiti

The collection and dissection of wild mosquitoes have been used to determine filarial endemicity ever since the classical discovery by Manson in 1878 of the role mosquitoes play in the development of filarial parasites. This method has been particularly useful in detecting the species of mosquitoes which are the important natural vectors. Natural infection rates in the South Pacific area have been reported by O'Connor (1923), McKenzie (1925), Byrd *et al.*, (1945), Jachowski and Otto (1952), and Rosen (1955), for the areas in which they worked.

In attempts to evaluate changes in mosquito populations and in the rates of infection of the mosquitoes with *Wuchereria bancrofti* during the filariasis control program in Tahiti, several procedures of collecting mosquitoes for dissection have been practiced. Edgar (Beye *et al.*, 1952) made collections twice a month, of 10 minutes each, at three biting stations in the experimental zones under study. It was later considered that this method provided insufficient information to evaluate an area and the schedule was changed to collections once a week, for 20 minutes each, at 10 biting stations selected approximately equal distances apart in each of the six experimental districts (Kessel *et al.*, 1954). This method was followed for one year and seasonal data were obtained. It was found to be cumbersome and expensive, however.

These preliminary collection trials led to the development of the "Intensive Survey Method" here described in detail as a possible standard procedure for the collection of mosquitoes to be dissected and examined for filaria larvae. Preliminary reports were made by Kessel (1953) and Kessel *et al.*, (1954).

Previous studies by Rosen (1955) have indicated that of the several species of mosquitoes found in Tahiti, *Aedes polynesiensis* Marks is the most important vector of the nonperiodic *W. bancrofti* in this area. Consequently, in the present study, this survey method has been applied to this species only. It could be adapted equally well, however, to other species of mosquito by suitable modifications of the collection procedures.

COLLECTIONS OF MOSQUITOES

Since no successful trap for the day-biting *A. polynesiensis* is known, it has been necessary to capture the mosquito as it comes to human bait. Capture has been accomplished by utilizing an aspirator with a plastic tube similar to that recently described by Burton (1954). The mosquitoes are taken up by suction as they alight to feed on the bait. Collections are made for a period of 10 minutes

[1] Aided by U. S. Public Health Service Grant G 3811.

within ten meters of each habitation in the area where the survey is being made. Human habitations include all dwellings with their associated buildings, churches, stores, schools and other buildings. A conscious effort is made to select the shadiest and most protected location within this area to insure the greatest likelihood of catching mosquitoes if any are present. The full 10 minutes of effort is spent at every collection "station" even though there may be the subjective impression that mosquitoes are absent. Thus every area of human habitation is sampled irrespective of the physical nature of the surroundings. For example a hot, dry area would not be expected to have as many mosquitoes as a cool, humid area. However, by making the effort of collection the same for all areas, the quantitative measurement of mosquito populations is modified in proportion to the extent of each type of area present within the district. The calculation of the number of mosquitoes per minute is readily done and gives an "average" for the "biting" mosquito population of the entire area. Thus, the statistical difficulty of determining valid sampling points can be avoided, and it is possible to compare the results from one area with those of another. Another important feature of the intensive survey method is that the collections are not made in sequence at adjacent locations. Rather, a random procedure is established. Every second, third or fourth location is skipped, to be collected at a later time. In general, the locations of collections are separated by a distance of at least 150 meters. This randomizing method insures that all areas of the district under study will have collections made at different times of the day, or on different days. Therefore, if the weather conditions have changed in the course of the survey, all areas will be represented in the collections made under the different conditions. Mimeographed maps of the area showing the location of all buildings within the district are used and, as each location is collected, it is marked with colored pencil, thus insuring that no location is missed as a result of this randomizing procedure.

During the collection period, the human bait bares his body to the waist and sits quietly, while the catcher circles slowly around, sucking up the mosquitoes into the tube as they alight. Usually only the bait and the catcher are present but it is unavoidable that occasionally two or three inquisitive children stand about watching. As the mosquitoes are caught, they are identified as to species and placed in cotton-stoppered test tubes containing a strip of damp paper toweling. All the mosquitoes from a single station are placed in the same tube, which is then identified by a strip of paper giving the number and species of mosquitoes and the location of the capture station.[2] Complete records are kept of the times of collection, locations, weather conditions, numbers of mosquitoes and species present at all collection points. Experience has shown that a two man collecting team consisting of bait and catcher is able to collect 30 to 36 stations in one day, with approximately 5 minutes travel time between

[2] For the filariasis work in Tahiti, maps have been prepared showing the location of every building in each kilometer of the circumferential road. The location of any house and the mosquitoes captured there are adequately described by giving the kilometer and house number, i.e. km 29 H 6.

collection points. With two collecting teams of 4 men it is possible to finish one round of collections in the average district in Tahiti in less than three days.

DISSECTIONS OF MOSQUITOES

An integral feature of the intensive survey method is the dissection of the mosquitoes captured by the above described method, a short time after capture. Thus the information on filarial infection obtained from the dissections may be related to the relative abundance of the mosquitoes and the exact location of capture. In Tahiti, a dissection team accompanies the collection teams into the districts and completes the dissections within three to six hours after the mosquitoes are collected. In a few instances, mosquitoes have been held overnight under refrigeration and dissected the following day, but this has been avoided whenever possible. Through the cooperation of the district chiefs, a room with table and chairs is made available in the various districts for temporary use as a field laboratory. All other equipment is transported from area to area by truck.

After the mosquitoes are captured they are taken in labeled tubes to the field dissection center. It has been the practice for all members of the intensive survey team to capture mosquitoes in the morning and to detach two members in the afternoon for the dissection work. This schedule has been flexible depending upon the number of mosquitoes captured in the morning. If great numbers are being caught, four persons will engage in dissections; if only a few are being captured, the dissection work will not begin until late afternoon. Although such practice requires that all members be trained in both collection and dissection techniques, it allows for an automatic adjustment of the flow of work by varying the personnel to meet the demands of the dissection program. A change to collection is also appreciated as a relief from the tiring tedium of the dissection work, with consequent increased accuracy in the dissection results.

The cotton plugs in the test tubes are replaced with one slightly dampened with chloroform or ether. This is done one tube at a time to avoid over-anesthesia, which will kill the filarial larvae if prolonged. As soon as the mosquitoes are moribund, they are placed in a petri dish and the wings and legs removed under the low power magnification of the dissecting microscope by means of two pairs of jewelers' forceps. The individual mosquitoes are then placed on a microscope slide in a drop of physiological saline. All the mosquitoes from a single station, i.e., in a single tube are handled as a group, with the slides placed in a vertical row on the table. The first slide in each row is placed on top of the identification slip which accompanied the test tube, and the information on the slip will apply to all the mosquitoes in that row. All mosquitoes in the tubes are prepared in this way if there are 10 or less. In cases where more than 10 are present, a random selection of 10 mosquitoes is prepared for dissection. This limitation of numbers dissected was found necessary to insure that the dissections were completed within a reasonable period of time and that large numbers of mosquitoes from two or three stations did not unduly "weight" the results.

The anesthetizing, removal of wings and legs, and arrangement of the mosquitoes on the vertical rows of slides are done by the first member of the dis-

section team. The second member of the team then takes each slide, being careful to maintain the labeled groups, and with dissecting needles divides the mosquito into head, thorax and abdomen, placing each portion of the body in a separate drop of saline on the same slide. This procedure is standardized so that the thorax is placed in the central drop. The thorax is gently divided into two or three large pieces, but is not completely dissected at this time. The head and abdomen are not divided, although care is taken to see that they are immersed in the saline. Each mosquito is handled in this manner until a group of 40 to 60 has been prepared in this preliminary fashion, or a total time of 30 minutes has elapsed. It has been found that during this period, most of the filariae of the infectious stage will have emerged from the tissues intact and frequently the younger stages also will drop out. This facilitates the detection of infected mosquitoes and the recovery of intact third stage filarial larvae. An additional advantage is the absorption of fluid by the mosquito tissues, making them softer and easier to dissect. After this waiting period, the slides are taken by the first member of the team and the three portions of each mosquito carefully teased apart under the dissecting microscope. Each portion is macerated until no particle is large enough to conceal any stage of development of the filarial worm. During this process the slide is carefully examined for the presence of infection in the mosquito. Each slide on which a mosquito positive for developing filarial larvae is found, is placed slightly out of line in the vertical row to indicate the presence of larvae. The slides are then carefully reexamined by the second member of the dissection team or by the field-team leader in order to be certain that no infection has escaped detection. It has been found that the dissection of 250–275 mosquitoes a day is the maximum that can be expected of 2 dissectors. The stage of development as outlined by Kobayashi (1940), the number of developing larvae and the location within the body of the mosquito are recorded. In addition, careful identification of the filarial larvae is made to distinguish the larvae of *W. bancrofti* from other filarial forms.[3] Since the location of capture of each mosquito is known, it is also possible to determine the house or houses where positive mosquitoes were taken. This information may in turn be correlated with the collection data with respect to the time of day, climatic conditions, etc.

RESULTS

A. *Mosquito collections.* The results from the standard collections of mosquitoes by the intensive survey method have been analyzed in three ways to obtain a measure of mosquito populations: (a) rate of capture, (b) per cent of stations with biting mosquitoes, and (c) per cent of stations with 10 or more biting mosquitoes. Although each of these can serve as a quantitative measure of mosquito populations, the data must be interpreted with care. It is particularly important to remember that owing to the method of capture all of these popula-

[3] In Tahiti, only two filarial parasites are known, *Wuchereria bancrofti* and *Dirofilaria immitis*. The latter species is separated on the basis of early stages developing only within the Malpighian tubules and the presence in third stage larvae of a single caudal papilla rather than 3 as seen in *W. bancrofti*.

tions refer to "biting" mosquitoes only. The numbers biting at any one time are determined by absolute mosquito population, age of population, previous exposure to suitable hosts, climatic conditions, and other factors. With the intensive survey method of collection at a large number of sampling points throughout a district, these uncontrolled factors are "averaged" and the result is a relative measure of the biting population at the time of the sample. This is considered satisfactory, as transmission involves only that portion of the entire mosquito population which is biting.

(a) *Rate of capture.* This is a common index for measuring mosquito numbers and is obtained by dividing the number of minutes spent in actual catching effort into the number of mosquitoes caught during that time. Jachowski and Otto (1953) used "⅓ man hour" as the unit of time. This is equivalent to 10 minutes of catching effort per station since they include both the catcher and the bait in the man-hour calculation. In Tahiti, the amount of time for capture by the intensive survey method has been standardized at 10 minutes per station, thus making the rates of capture in Tahiti comparable with the results from American Samoa.

The rate of capture per minute is an average figure for all collections in a district and includes both stations with no mosquitoes and those with very large numbers of mosquitoes. During the last three years, 64 intensive surveys have been completed in the 19 districts of Tahiti. This has involved the collection of 49,119 specimens of *A. polynesiensis* at 14,248 stations, with 10 minutes of collection at each station. Thus the capture rate during the period 1953–1955 was 0.35 mosquitoes per minute, or approximately 1 mosquito for every 3 minutes of catching effort. The range between different surveys has been from 0.03 to 1.27 mosquitoes captured per minute. Also it has been observed that during this same period there has been a reduction in the rate of capture from 0.42 per minute to 0.20 mosquitoes per minute. This is believed to reflect the efforts of the district inspectors who have been instructing the inhabitants in sanitation and the elimination of mosquito breeding sites.

The purpose of catching these mosquitoes was to obtain a sample for examination for infection with filaria larvae. The collection points were selected on the basis of likelihood of infection and not on the basis of the presence of large numbers of mosquitoes. In American Samoa, Jachowski and Otto (1952) reported the capture of 1,734 mosquitoes at 121 stations of 10 minutes each. This would give a calculated rate of capture of 1.4 mosquitoes per minute. Since approximately one-half of the collection stations were located in "bush" areas, it is not surprising that the rate is higher than that reported here for Tahiti utilizing the intensive survey method.

(b) *Percentage of stations with biting mosquitoes.* A measure of mosquito population which avoids certain difficulties inherent in the capture rate is one based upon the number of collection points where mosquitoes are captured during the 10 minute collection period. The percentage of points can be determined from the records of the intensive survey since attempts to capture mosquitoes within a reasonably short period of time are made at many similar sampling points

which are widely separated throughout the district. This gives, in effect, a measure of the geographic distribution of the mosquito population. Various workers including Byrd, St. Amant *et al.* (1945), and Jachowski and Otto (1953) have indicated that the dispersal range of *A. polynesiensis* is probably less than 100 meters. Therefore, the capture of this mosquito would indicate a breeding site within this radius and one is justified in considering this figure as an index of population. In the three years of experience previously mentioned only 46 per cent of all the locations where attempts were made to capture mosquitoes actually had one or more mosquitoes taken during the 10 minute period. This figure in different surveys, has ranged from a high of 81.2 per cent to a low of 16.4 per cent of the stations with mosquitoes. It has been noticed that there is less variation in the percentage of houses with mosquitoes than in the rate of capture when one compares the results from several surveys in the same area. This is probably because the presence of large numbers of mosquitoes at one or several stations does not influence the results, or introduce an unusual bias. From the standpoint of disease transmission and mosquito control, this is a valuable index because it is directly related to the actual presence of mosquitoes close to human habitations. Belkin (1954) has suggested a somewhat similar adult index whereby the rate of capture is combined with the number of stations where mosquitoes are taken.

(c) *Percentage of stations with 10 or more mosquitoes.* A refinement of the previously mentioned indices of mosquito population is the determination of the percentage of houses with 10 or more mosquitoes captured during the 10 minute period. This introduces a semi-quantitative feature to the previously described geographic or distributional index. During the surveys in Tahiti, the average percentage of houses with 10 or more mosquitoes captured during the 10 minute collection period has been 10 per cent with a range of 0 to 36.3 per cent. In the following list, the rate per minute, percentage of houses with mosquitoes, and the percentage of houses with 10 or more mosquitoes captured during the capture period are shown.

The survey results obtained in 4 districts of Tahiti in July, 1954, are presented below and compare the rate of mosquitoes captured per minute and the per cent of houses (a) with no mosquitoes, (b) with less than 1 per minute, and (c) with more than 1 per minute.

District	Population	Mosq./Minute	No Mosq.	Percentage of Houses with	
				Less than 1/minute	More than 1/minute
Afaahiti	567	0.55	38.5%	42.3%	19.2%
Faaone	439	0.65	29.4	44.0	26.6
Tiarei	302	0.77	18.9	47.7	33.4
Mahaena	182	1.13	37.5	34.1	28.5

In the district of Afaahiti, 38.5 per cent of the houses had no mosquitoes. This means that out of a population of 567, approximately 228 were not exposed to bites from *A. polynesiensis* during the survey period, and only 19.2 per cent,

or 109 persons, were subject to attacks at a rate greater than 1 per minute. Similar calculations show that only 117 persons are bitten at this rate in Faaone, 100 in Tiarei, and 52 in Mahaena. Thus more people are exposed to a higher number of mosquitoes in Faaone, even though the rate of capture per minute is greatest in the district of Mahaena. Efforts to combine the various indices into a single useful index have been only partially successful. It is apparent that the rate of capture does not completely represent the "transmission" hazard in a district.

B. *Mosquito dissections.* The results obtained from the dissections of the mosquitoes captured by the collection methods described above are (a) percentage of mosquitoes infected with developing stages of *W. bancrofti*, either including all stages or limiting the result to infective stages only; (b) number of larvae per dissected mosquito; and (c) percentage of collection stations from which mosquitoes positive for infection were captured.

(a) *Percentage of mosquitoes infected with* W. bancrofti. This is determined by the actual number of mosquitoes observed to have any developmental stages except microfilariae, divided by the total number of mosquitoes dissected. This figure is the most frequently reported estimate of the extent of filarial infection in the mosquitoes of any area. Iyengar (1954) has summarized the information available on the mosquito infections in the South Pacific area. It is not always possible, however, to compare the results of the various workers unless the collections have been made in the same manner. Mosquitoes captured at a single location or in only a few locations may produce very different results depending upon the amount of filarial infection to which the mosquito population was exposed during the previous two weeks.

Some workers, Symes in Fiji (personal communication) and Rosen (1955), state that the percentage of mosquitoes carrying third or infective stage larvae of *W. bancrofti* is more indicative of the degree of filarial infection in relation to transmission, since it is recognized that not all ingested microfilariae successfully develop to the infective stage (cf. Kartman, 1954). In the work in Tahiti, it is believed that the inclusion of all stages of development in any index makes the attainment of established control goals more difficult to reach and that any error would be on the side of safety.

(b) *Number of larvae of* W. bancrofti *per dissected mosquito.* In order to obtain a quantitative measure of the intensity of the filarial infection in the mosquito, it is necessary to determine the average number of larvae per positive mosquito. This is found by dividing the total number of larvae by the number of positive mosquitoes. However, since the number of positive mosquitoes in a population is a factor in the transmission hazard, it has been found expedient to combine the two figures to obtain the average number of larvae in the examined population as follows:

$$\text{No. of positive mosquitoes per standard population} = \frac{\text{No. of positive mosquitoes}}{\text{No. of mosquitoes dissected}}$$

$$\text{Average no. of larvae of all stages per positive mosquito} = \frac{\text{Total no. of larvae}}{\text{No. of positive mosquitoes}}$$

These, when multiplied, reduce to:

$$\frac{\text{Total no. of larvae}}{\text{No. of mosquitoes dissected}} = \begin{matrix}\text{Larval Density} \\ \text{or} \\ \text{Average no. of larvae per} \\ \text{dissected mosquito}\end{matrix}$$

In Tahiti it has been found that the number of developing larvae in the mosquito population reduces markedly as the people of the district are treated with diethylcarbamazine. This figure can serve, therefore, as a measure of the filarial infection within the district examined. Kessel (1955) utilized the information obtained by the intensive survey method and calculated a "potential transmission index". The average number of *W. bancrofti* larvae in the dissected mosquitoes multiplied by the rate of mosquito capture per minute has proved to be a satisfactory means of evaluating the progress of a filarial control program.

(c) It has also been possible to calculate the percentage of stations in the

TABLE 1

Results of a typical district before the utilization of control measures

Number of collecting stations	112
Number of mosquitoes collected	615
Number of stations with mosquitoes	75 or 66.9%
Number of stations with 10 or more mosquitoes	20 or 17.8%
Number of stations with mosquitoes positive for larvae of *W. bancrofti*	29 or 25.8%
Number of mosquitoes caught per minute	0.54
Number of mosquitoes dissected	422
Developmental stage larvae present in mosquitoes	56 or 13.2%
Infective stage larvae present in mosquitoes	32 or 7.6%
Density of developmental stage larvae per dissected mosquito	0.74
Density of infective stage larvae per dissected mosquito	0.19

TABLE 2

Results of a typical intensive survey in a district following recommended control measures, including treatment with diethylcarbamazine

Number of collecting stations	98
Number of mosquitoes collected	107
Number of stations with mosquitoes	30 or 30.6%
Number of stations with 10 or more mosquitoes	2 or 2.0%
Number of stations with mosquitoes positive for *W. bancrofti* larvae	1 or 1.0%
Number of mosquitoes per minute	0.109
Number of mosquitoes dissected	65
Developmental stage larvae present in mosquitoes	1.0 or 1.5%
Infective stage larvae present in mosquitoes	0.0 or 0.0%
Density or average number of all stages of *W. bancrofti* larvae per dissected mosquito	0.061
Density or average number of infective stage larvae per dissected mosquito	0.0

area at which positive mosquitoes were found. Collecting stations where more than one mosquito are found to be positive are counted only once in making this calculation. This avoids weighting the results, which will occur if numerous mosquitoes are captured at a house inhabited by a microfilarial carrier, and where the opportunity to find infected mosquitoes is high. This is the number of positive collection stations per 100 stations, or 1,000 minutes of catching effort.

DISCUSSION

The various calculations presented above represent a few of the different types of information which can be determined from the data obtained by the intensive survey method. The exact value of all of these indices is not known at present and must be further tested. It is encouraging to note, however, that by the current standardization of collections and dissections of the mosquitoes, the information gained takes on new significance and can readily be used as a measure of the intensity of filarial infection within a population.

Furthermore, the intensive survey method has proved to be an independent check upon the work of the inspectors who distribute the diethylcarbamazine. Blood surveys as reported by Kessel (1955) have been made once a year in districts of Tahiti since the filariasis control program began and these have given information concerning the reduction of microfilarial infection in man. A blood survey provides a satisfactory index but requires longer and is more expensive than an intensive mosquito survey, which for most districts, can be completed within a week, and has proved to be sufficiently sensitive to serve as a supervisory yard stick. In several instances, the first indication that a drug treatment program was not being executed correctly came through the intensive mosquito survey, the results being later confirmed by a blood survey. The inspectors in each district have become more alert since the introduction of the intensive survey, which also aids them in detecting the locations where positive mosquitoes are to be found. A follow-up blood collection of the people in the positive houses often enables the inspector to find persons still carrying microfilariae who may have escaped treatment.

The collection of mosquitoes at each house in the district not only provides information on the distribution and numbers of mosquitoes present, but offers an opportunity for public education in filariasis control by the persons collecting the mosquitoes. This has proved to be a real benefit as previously there were many people who did not seriously believe that the mosquito was involved in the transmission of the disease. However, after explanation by the trained team members while watching the dissections and observing the living worm, the leaders and people of a district accept more readily instruction in principles of mosquito control.

SUMMARY

A method of collection and dissection of mosquitoes, particularly *Aedes polynesiensis* in Tahiti, is described. This "Intensive Survey Method" provides a distributed sample of mosquitoes for dissection, while simultaneoulsy the follow-

ing indications of mosquito densities can be determined: (1) the number of mosquitoes captured per minute of capture effort; (2) the percentage of habitations at which mosquitoes were captured; (3) the percentage of houses where 10 or more mosquitoes were captured during the 10-minute capture interval. By dissection and examination of these randomly collected samples of mosquitoes, the percentages of mosquitoes with developing and/or infective larvae of *Wuchereria bancrofti* are determined. In addition, the number of developing *W. bancrofti* larvae per dissected mosquito can be calculated as well as the percentage of houses with positive mosquitoes. The interpretation of the data obtained is discussed briefly along with the advantages and disadvanrage of this method.

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ACTH AS AN ADJUNCT TO THE TREATMENT OF LOAIASIS

JAMES H. THOMPSON

Department of Medicine, University of California School of Medicine, San Francisco, California

This single case report is of interest because of the marked symptomatic relief provided by administration of ACTH during treatment of loaiasis with diethylcarbamazine (Hetrazan). ACTH as an adjunct in the therapy of loaiasis has not been described previously.

The use of corticosteroids in the treatment of filariasis was noted by McFadzean (1953). He indicated that most of the reported improvement in elephantiasis was produced by bed rest and elastic bandages, rather than by a specific effect of corticosteroids. Markell (1955) pointed out that administration of cortisone to patients with elephantiasis due to *Wuchereria bancrofti* was followed by diuresis and an increased number of microfilariae in the blood stream. Both effects were ascribed to a lessening in the inflammatory reaction around the adult worms which allowed freer lymphatic drainage from the swollen limb and greater access by the microfilariae to the lymphatics and the blood stream.

CASE REPORT

D. F. is the 12-year-old daughter of a missionary whose home is in Gaban, French Equatorial Africa. The incidence of *Loa loa* is high in the area. All members of the patient's family harbor this parasite, as indicated by the finding of microfilariae in the blood stream.

The patient had been quite well and was attending school in San Francisco while her parents were on leave in this country. During cool weather she was asymptomatic. On a visit to Southern California, however, she was exposed to warm weather which produced a severe skin reaction and numerous "Calabar swellings," particularly over the exposed portions of the lower legs, ankles, forearms and hands. Because of this acute reaction she was hospitalized for treatment.

On physical examination the patient weighed 108 pounds. Her temperature was 101.6°F. She had "Calabar swellings," about 12 in number, over the dorsum of both hands and feet and on the forearms, lower legs and left cheek beneath the eye, as well as a generalized mild urticarial rash. The tip of the spleen was palpable below the left costal margin. There were a few small, firm, non-tender, movable lymph nodes in the inguinal and cervical regions. No other abnormalities were found.

Administration of Benadryl, 25 mg. three times daily, and the application of Ace bandages around the joints relieved the general urticarial rash and partially relieved the pain in the hands and feet. Specific treatment with diethylcarbamazine was begun on April 3. On April 4 a severe exacerbation of the "Calabar swellings" caused almost unbearable pain, which was not relieved by Benadryl, Ace bandages and elevation of the feet. Initiation of ACTH therapy at this time resulted in almost immediate relief of swelling and pain. The combination

TABLE 1

Treatment of a case of loaiasis with a combination of diethylcarbamazine and ACTH

April	3	4	5	6	7	8	9	10	11	4/12 to 5/11
Diethylcarbamazine, mg.	50	100	200	300	300	300	300	300	300	300
ACTH Gel, I.U.			15 twice daily	15 twice daily	10 twice daily	15 once daily	10 once daily	5 once daily	0	
Max. Temp., °F.	101.6	101.4	101.4	100.0	98.6	98.6	98.6	98.6	98.6	

of diethylcarbamazine and ACTH was continued through April 10. The doses and details of treatment are summarized in Table 1.

At the time of discharge from the hospital on April 11 the patient was almost free of symptoms. She continued to take diethylcarbamazine, 300 mg. daily, without other medications, for one month. The "Calabar swellings" diminished in size and tenderness, and after 3 weeks of treatment disappeared. The patient stated that she now feels stronger than she has for several years, and her parents believe that she is more active and alert than in the past.

DISCUSSION

In this case of loaiasis the adjunctive use of ACTH resulted in the dramatic relief of symptoms exacerbated by treatment with diethylcarbamazine. Because the occurrence of further cases in this locality is highly unlikely, we have reported on this single instance in the hope that these findings can be confirmed in other parts of the world where the disease is more common. It is also suggested that ACTH might be of similar value in alleviating the severe skin reaction encountered in many cases of onchocercosis during therapy with diethylcarbamazine.

We should also like to draw attention to the interesting observations of Markell (1955), in particular his statement that the microfilariae in the blood of patients with Bancroft's filariasis are greatly increased in number following administration of cortisone. It occurred to us that cortisone might be used as a provocative test in cases where filariasis is suspected, although microfilariae cannot be demonstrated in the blood. In such instances administration of cortisone over a 2- or 3-day period might allow microfilariae to penetrate the lymphatic barriers in sufficient numbers to be demonstrated in blood smears and a diagnosis made. Use of cortisone for such a short period would certainly not be harmful, providing the patient was kept under medical observation.

CONCLUSION

ACTH, and probably other corticosteroids, appears to be a useful adjunct in the treatment of loaiasis with "Calabar swellings," especially during the acute exacerbation of symptoms caused by treatment with diethylcarbamazine. Corticosteroids may also be of symptomatic benefit in the treatment of

onchocercosis with this agent. On the basis of Markell's observation that patients with Bancroft's filariasis show a great increase in the number of microfilariae in the blood during therapy with corticosteroids, it is suggested that cortisone might also serve as a provocative test in questionable cases of filariasis.

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BOOK REVIEWS

La Lèpre, by ROLAND CHAUSSINAND, Chef du Service de la Lèpre a l'Institut Pasteur. 2nd ed., 310 pp. illustrated, with 18 in color. Paris, Expansion Scientifique Française. 3,800 fr.

The author, who worked for fifteen years as a leprologist in Saigon and now is in charge of the Leprosy Service at the Pasteur Institute in Paris, gives us in this one volume a work on leprosy which covers admirably well the whole subject in concise form.

The chapter on history contains fascinating information, especially of the Indian and Greco-Roman periods. The chapter on the lepra bacillus is particularly interesting, dealing with its appearance in various forms, its culture, staining, and animal inoculation. Leprosy in animals, especially rat and buffalo, is well covered. The chapter on etiology gives important information regarding penetration of the bacilli, its emission, and the possibilities of transmission. When discussing the immunology, the author stresses the importance of the lepromin test and gives detailed information regarding its preparation. The clinical aspects and diagnosis of leprosy are dealt with in close connection with its histopathology, illustrated by more than one hundred instructive photographs, and extremely well covered. Excellent, also, is the presentation of the difficult and controversial question of classification. In the chapter on prophylaxis the author stresses the antagonism between tuberculosis and leprosy and discusses BCG vaccination and vaccination with microbacterium Marian. Therapy includes modern chemotherapy; management of reactions; eye and other special lesions; treatment of complications; orthopedic and plastic surgery.

Throughout the whole book the author combines his own extensive experience with perfect knowledge of the world literature on this subject. He advocates a modern attitude toward this disease, deplores the medieval legal restrictions still in force in some areas against patients suffering from leprosy, and stresses the main factors essential for any leprosy control problem, namely, early recognition, adequate treatment, and liberal regulations, as advocated also by the modern leprologists in this country. An excellent work and a pleasure to read. Recommended for neophyte, as well as expert.

PAUL FASAL

The History of the School of Tropical Medicine in London (London School of Hygiene and Tropical Medicine Memoir No. 11), by SIR PHILIP MANSON-BAHR. 329 pp. illustrated. London H. K. Lewis & Co., Ltd., 1956. £2.10s.

This memoir is divided into four sections preceded by a preface in which Dr. Charles Wilcocks, Director, Bureau of Hygiene and Tropical Diseases, gives a most satisfactory biography of the author.

Section I: Historical, presents a survey of tropical medicine in 1898 which shows the times were ripe for the establishment of such a school. The author then describes the small beginnings from which, due to Sir Patrick Manson's firm lead, the backing given him by Mr. Joseph Chamberlain, Secretary of State for the Colonies and the munificent gift of the Rockefeller Foundation, the imposing school of today has evolved. As the history of the school proceeds Sir Philip keeps the reader abreast of the continuing development and great expansion of the field of tropical medicine, due in no small part to the unselfish devotion and unremitting labor of the men connected with the school.

In Section II: Biographical, Sir Philip, whose "words, like so many nimble and airy servants trip about him at command" is at his best as he knew well so many of the founders, directors, deans, physicians, lecturers, technicians and others, of whom he writes so vividly, showing how

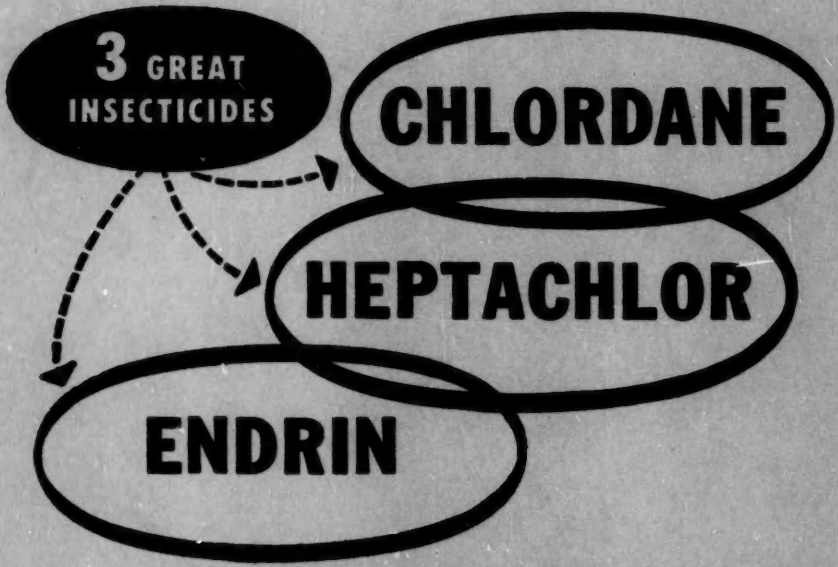
> "their work continueth,
> Broad and deep continueth,
> Greater than their knowing."

Section III consists of a "Diary of the School", 1899–1949, drawn to a great extent from the records of the Seamens' Hospital Society to which great credit is due not only for the founding of the School but for its growth.

Section IV: Appendix, lists the research expeditions undertaken by the School, the collected papers of the School, the prize winners, and gives the syllabus of lectures at the School as set out in 1913. The last chapter is a resumé of tropical medicine in practice at the Hospital for Tropical Diseases, 1920–1939.

The book will be of especial interest to all those who have participated in the work of the School in any way and present workers in tropical medicine who are interested in their illustrious forerunners will find much pertinent information presented in Sir Philip's inimitable fashion.

FLORENCE M. FROST



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